ACTIVATION OF RAT LIVER ALCOHOL DEHYDROGENASE BY DEOXYCHOLIC ACID

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1. Introduction

The presence of the detergent Triton X-100 during homogenization increases the activity of alcohol dehydrogenase in the supernatant of rat liver homogenates [1]. We have tested the effect on the enzyme of a number of non-ionic and ionic surface active compounds, chiefly bile acids. These latter compounds were particularly taken into consideration as they are physiological metabolites and alcohol dehydrogenase plays a role in their biosynthesis in the liver cell [2–4]. Deoxycholic acid was found to be a considerable activator of the enzyme, and some data are given on the characterization of this steroid as a modifier of the enzyme protein.

2. Materials and methods

Livers from Sprague-Dawley male albino rats weighing 150–200 g were used for the preparation of alcohol dehydrogenase. The enzyme purification was carried out by ammonium sulphate precipitation, DEAE-Sephadex A-50 chromatography and gel filtration on Sephadex G-100, according to Markovič et al. [5]. Minimal final specific activity of the preparations was 2.2 μmol/min/mg protein, with a 50-fold purification. Gel electrophoretic analysis, carried out according to Lutstorf et al. [6] with specific staining for alcohol dehydrogenase, indicated the presence of only one band.

The alcohol dehydrogenase activity was determined at 30°C, by recording the change of optical density at 340 nm with a Gilford 2400 spectrophotometer, in a test mixture having the following composition: 50 mM triethanolamine buffer, pH 7.6; 5 mM EDTA; 0.2 mM NADH, 2 mM propionaldehyde, in a final volume of 1 ml. The rate of the backward reaction was measured according to Markovič et al. [5], in a test mixture with the following composition: 100 mM glycine buffer, pH 10.0; 1.5 mM NAD^+; ethanol or cyclohexanol or 3β-hydroxy-5β-androstane-17-one at the concentrations indicated in fig.2, in a final volume of 1 ml. The bile acids were added to the assay mixture as sodium salts. Proteins were determined by a biuret method [7] or by u.v. extinction [8].

3. Results and discussion

Table 1 shows the effect of some surface active agents on the specific activity of purified rat liver alcohol dehydrogenase. Deoxycholic acid has a remarkable effect, activating the enzyme at levels below 3 mM, that is the critical micellar concentration [9]. The effect of deoxycholic acid is reversible, activation being abolished by simple dilution of enzyme samples preincubated with the bile acid. Other surface active agents have no effect or else they inhibit: only Triton X-100 shows a slight activating action, as already observed [1].

Deoxycholic acid activates alcohol dehydrogenase also in the soluble fraction of rat liver homogenate: it however does not increase the amount of extracted enzyme during homogenization. Other bile acids – like cholic, taurocholic and chenodeoxycholic – are active as well on purified rat liver alcohol dehydrogenase, but they show a slighter activating power than deoxycholic acid; lithocholic acid is ineffective (fig.1).

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Table 1
Effect of deoxycholic acid (DOC) and some surface active agents on the activity of purified rat liver alcohol dehydrogenase (ADH). Substrate: propionaldehyde, pH 7.6

Addition	Concentration (mg/ml)	ADH specific activity (μmoles/min/mg prot.)	ADH relative activity
–	–	2.28	1.00
DOC. Na	0.08	3.15	1.38
	0.16	3.99	1.75
	0.24	4.56	2.00
	0.40	5.93	2.60
Triton X-100	0.2	2.46	1.08
	0.5	2.85	1.25
	1.0	3.42	1.50
	2.0	3.65	1.60
Brij-58	0.2	2.28	1.00
	0.4	2.28	1.00
	0.8	1.60	0.70
Tween 80	0.2	2.01	0.88
	0.4	1.71	0.75
	0.8	1.60	0.70
Palmitate. Na	0.0028	1.42	0.62
	0.0280	1.28	0.56

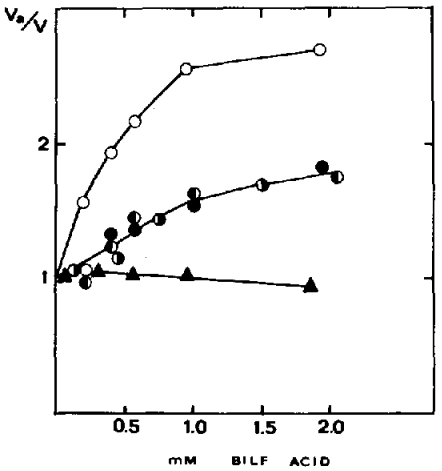

Fig.1. Effect of some bile acids on the activity of purified rat liver alcohol dehydrogenase. v = Reaction rate in absence of bile acids; v_a = reaction rate in presence of the indicated concentrations of bile acids. Substrate propionaldehyde, pH 7.6. (○) deoxycholic acid; (●) cholic acid; (◐) chenodeoxycholic acid; (◑) taurocholic acid; (▲) lithocholic acid.

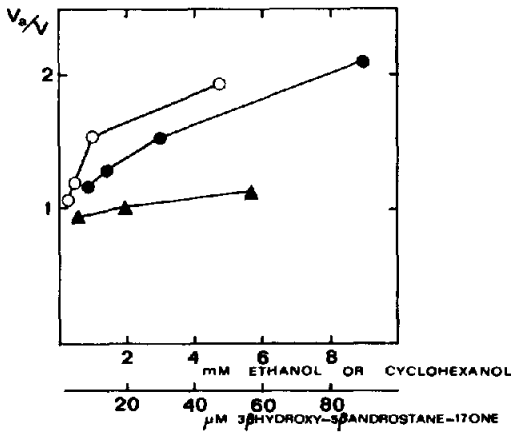

Fig.2. Effect of the substrate concentration on the activation by deoxycholic acid (DOC) of purified rat liver alcohol dehydrogenase. v = Reaction rate in absence of DOC; v_a = reaction rate in presence of 0.96 mM DOC. Substrates: (○) cyclohexanol; (●) ethanol; (▲) 3β-hydroxy-5β-androstane-17 one; pH 10.0.

Activation by deoxycholic acid does not depend upon the buffer used in the test. It depends however on the substrate type and concentration: with ethanol or cyclohexanol as substrates activation increases with substrate concentration, with the steroid alcohol 3β-hydroxy-5β-androstane-17-one no effect is found on the reaction rate (fig.2).

It is difficult to explain at present the functional significance of activation of rat liver alcohol dehydrogenase by some bile acids. The main physiological role of this enzyme has long been an open problem, as it is active on a number of different alcohols, including ethanol, retinol and cyclic alcohols. The importance of alcohol dehydrogenase in the oxidation of ethanol produced by intestinal microflora has been pointed out by Krebs and Perkins [10]. However from the work of Waller et al. [11] and successively of Okuda et al. [2–4] it results with evidence that a very important function of hepatic alcohol dehydrogenase is also the oxidation of steroid alcohols, specially of 3α, 7α, 12α, 26-tetrahydroxy-5β-cholestane, during the biosynthesis of cholic acid from cholesterol. It must be pointed out that – unlike in other species such as man and horse – only a single form of alcohol dehydrogenase has been found in rat [4,12]: the absence of isoenzyme forms in rat liver seems to depend on the participation of a single type of subunit in the assembling of the enzyme dimer [13]. It must also be emphasized that in the rat, unlike other species, deoxycholic acid deriving from action of intestinal microflora is converted to cholic acid as soon as it reaches the liver and is not present in the rat bile [14]. Finally it must be added that, according to our preliminary data, different responses to deoxycholic acid are obtained with alcohol dehydrogenase from liver of different animal species.

The eventual functional role played by bile acids as modifiers of liver alcohol dehydrogenase requires therefore further investigation and is under scrutiny.

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