ALDEHYDE DEHYDROGENASE IN HUMAN BLOOD

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1. Introduction

Oxidation of ethanol in the liver leads to the formation of acetaldehyde which is potentially more toxic than ethanol [1]. Korsten et al. [2] have demonstrated that following administration of ethanol, blood acetaldehyde concentrations are elevated to a greater extent in alcoholics (42.7 nmol/ml) than in non-alcoholics (26.5 nmol/ml) which suggests that in alcoholics either the rate of formation or of catabolism of acetaldehyde is altered. Thus, acetaldehyde seems to be implicated in alcoholism-related disorders (Korsten et al. and Raskin [2]).

This paper is the first report of occurrence of aldehyde dehydrogenase (EC 1.2.1.3) in blood. In the rat high aldehyde dehydrogenase levels in organs other than liver have been found [3]; no aldehyde dehydrogenase activity was, however, detected in the blood. The disappearance of acetaldehyde upon incubation with rat blood has been attributed to irreversible binding of acetaldehyde to hemoglobin [4]. Recently, two aldehyde dehydrogenases differing greatly in kinetic properties were isolated here from human liver [5]. Using human liver enzymes as markers, samples of blood (the only readily available human tissue) from a number of individuals with no known alcoholic history were examined. The results demonstrating and identifying aldehyde dehydrogenases in human blood are presented in this paper.

2. Materials and methods

Blood was collected and fractionated into plasma, membranes and intracellular fluid as described by Hanahan and Ekholm [6] except that EDTA treated (not heparinized) vacutainer tubes were used and 1 mM EDTA plus 0.1% 2-mercaptoethanol were incorporated into all buffers to stabilize aldehyde dehydrogenase. Membranes were dissolved by overnight incubation at 4°C in 0.26% w/v sodium deoxycholate also containing 1 mM EDTA and 0.1% 2-mercaptoethanol. An attempt was made to determine aldehyde dehydrogenase activity in 0.09 M pyrophosphate buffer pH 9.0 containing 450 μM NAD and 13.5 mM propionaldehyde in cuvettes of 1-cm light path and 3-ml volume. The reaction was started by addition of enzyme and followed at 340 nm and 25°C on a Varian 635 double beam recording spectrophotometer at 1:10 expanded scale. The control contained all components of the assay except propionaldehyde. Another control contained all components except the blood extract. The net rate of oxidation of propionaldehyde was obtained after subtraction of values obtained with both controls. In all blood fractions from 14 human subjects the isoenzyme content was simultaneously examined by electrophoresis. Kinetic measurements were made at varying concentrations of propionaldehyde with a Cary 118 Spectrophotometer at 1:50 scale expansion, using blood aldehyde dehydrogenase partially purified on DEAE Sephadex.

For electrophoresis 5-ml aliquots of blood fractions were concentrated by vacuum dialysis to less than 0.5 ml against Tris–HCl buffer, pH 7.6, containing EDTA and 2-mercaptoethanol. The dissolved membrane fractions were concentrated against sodium deoxycholate. Electrophoresis was done on starch gel in pH 5.5 citrate buffer (10 mM in the gel and 50 mM in the tank) at 200 V overnight at 4°C. The gels were developed in: 25 ml of 0.1 M pyrophosphate buffer

pH 8.3 containing 15 mg of NAD, 10 mg nitro-blue tetrazolium, 1 mg phenazine methosulphate and 13.5 mM propionaldehyde. The gels were developed by incubation at room temperature in the dark. Control gels were developed in the absence of substrate.

Protein was determined by the Lowry procedure [7] employing bovine serum albumin (fraction V, Sigma Chemical Co, St. Louis, MO) as a primary standard.

3. Results and discussion

An attempt was made to assay catalytic activity of blood aldehyde dehydrogenase in various blood fractions by employing a conventional NAD reduction method by measuring absorption at 340 nm. The net rate of propionaldehyde oxidation was variable (some values were positive, most were zero and some were even negative) and unrelated to aldehyde dehyrogenase content as simultaneously visualized on starch gels. Nevertheless using starch gel electrophoresis the presence of aldehyde dehydrogenase within the intracellular fluid was invariably detected. It is impossible at this stage to tell whether the enzyme also occurs in the plasma and membrane fractions. On one gel presence of an aldehyde dehydrogenase component in the membrane fraction was observed, this however might be a result of a contamination with the intracellular fraction. The plasma fraction contains a large amount of protein and is thus difficult to concentrate further for visualisation of the enzyme on gels. It is therefore not excluded that aldehyde dehydrogenase occurs in the plasma in small concentrations. Our current results indicate that aldehyde dehydrogenase occurs in the blood mainly in the intracellular fraction. The distribution of the enzyme within various blood cells is unknown. The difficulty to observe NADH formation in the presence of crude blood fractions may account for the reason why aldehyde dehydrogenase was never previously detected in blood.

A typical result obtained from electrophoresis of human blood aldehyde dehydrogenase is shown in fig.1. The intracellular fraction shows four aldehyde dehydrogenase components. The two major (E_4 and E_5 in fig.1) more anodal components, have never been described previously. The two more cathodal components have the same mobility as the liver enzymes E_1 and E_2. The aldehyde dehydrogenase detected by us in the membrane fraction migrated like the most anodal blood component. The zymograms of aldehyde dehydrogenase from the intracellular fraction of blood obtained from 14 volunteers were similar.

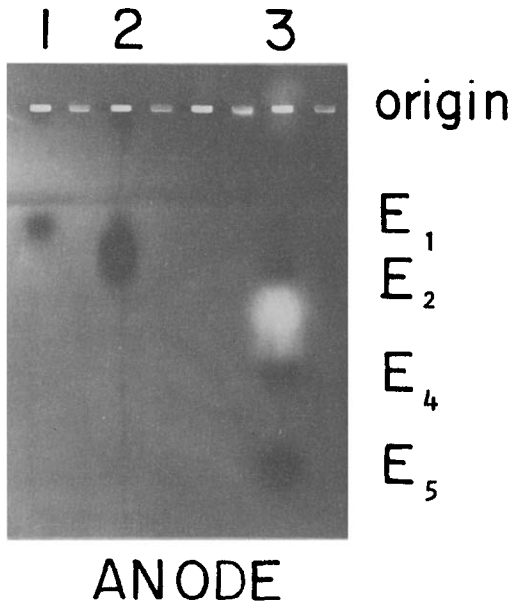

Fig.1. Electrophoretic separation of blood aldehyde dehydrogenase on starch gel. (1) Liver enzyme 1 (E_1) control; (2) liver enzyme 2 (E_2) control; (3) intracellular fraction of human blood showing four aldehyde dehydrogenase components: E_1, E_2, E_4 and E_5.

The two anodal enzymes (E_4 and E_5 in fig.1) were isolated by chromatography on DEAE Sephadex. The K_m value for the mixture of E_4 and E_5 (roughly 1:1 mixture by visual estimation on starch gel) at pH 9.0 with propionaldehyde was ca. 0.6 μM.

The total aldehyde dehydrogenase content in the cels from 20 ml of blood was estimated after isolating the enzyme by chromatography on DEAE Sephadex by employing NAD (450 μM), propionaldehyde (1.35 mM), or acetaldehyde (0.68 mM) in pyrophosphate buffer pH 9.0. The enzyme isolated had a total catalytic activity of 0.34 μmol/min; equivalent to 17 nmol/min/ml blood.

The reported non-alcoholic levels of blood acetaldehyde were 26.5 + 1.5 nmol/ml [2]. The value obtained here for blood aldehyde dehydrogenase activity as well as the low K_m for propionaldehyde indicate that blood aldehyde dehydrogenase may play a quantitatively important role in the clearance of blood acetaldehyde. Higher levels of blood acetaldehyde in alcoholics, among other possible reasons, may result from differences in the amount of blood aldehyde dehydrogenase or from differences in its isoenzyme composition. Easy accessibility of blood for experimental purposes will help in the study of this problem. The development of an assay system capable of determining the enzyme content in the presence of interfering blood components is essential.

Since blood aldehyde dehydrogenase activity is high relative to the reported steady state acetaldehyde levels [2], the enzyme may seriously interfere with acetaldehyde determination. Blood for determination of acetaldehyde must therefore be collected with suitable precautions.

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