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Short communication

Enhancement of alcohol dehydrogenase activity in vitro by acetylsalicylic acid

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Abstract

The interaction of acetylsalicylic acid with alcohol dehydrogenase was investigated. Horse liver alcohol dehydrogenase bound to a *p*-hydroxyacetophenone affinity column was eluted by acetylsalicylic acid. In vitro enzymatic activity of alcohol dehydrogenase in the presence of ethanol as a substrate was significantly increased by incubation with acetylsalicylic acid. These results suggest that acetylsalicylic acid has an affinity with alcohol dehydrogenase and enhances its activity.

Keywords: Acetylsalicylic acid; Alcohol dehydrogenase; *p*-Hydroxyacetophenone

1. Introduction

Acetylsalicylic acid, an anti-inflammatory drug, is known to influence metabolism of xenobiotica such as alcohol (Monroe and Doering, 2001; Weathermon and Crabb, 1999). Ethanol enhances acetylsalicylic acid-induced gastric mucosal damage and prolongation of the bleeding time (Deykin et al., 1982). Thus, alcohol beverage affects the efficacy of treatment with acetylsalicylic acid and its side effect profiles (Odou et al., 2001). However, little is known about the interaction of acetylsalicylic acid with alcohol dehydrogenase (ADH), an enzyme converting ethanol to acetaldehyde. Acetylsalicylic acid has been reported to show no effects on activity of ADH in the liver (Roine et al., 1990). On the other hand, acetylsalicylic acid inhibited ADH activity in gastric mucosa (Roine et al., 1990), although the mechanism of this action of acetylsalicylic acid is still unknown. Moreover, it still remains to be determined whether ADH activity is directly modulated by acetylsalicylic acid. In this brief study, effects of acetylsalicylic acid in vitro on enzymatic activity of ADH were examined using horse liver ADH. We found for the first time that acetylsalicylic acid has a direct enhancing effect on ADH activity.

2. Materials and methods

2.1. Materials

All chemicals were of reagent grade. Epoxy-activated Sepharose 6B and a PD-10 column were obtained from Amersham Pharmacia Biotechnology, Inc. *p*-Hydroxyacetophenone and acetylsalicylic acid were obtained from Wako Chemical Co. ADH from horse liver was purchased from Sigma. Molecular weight markers for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Owl Scientific, Inc. NAD was purchased from Boehringer Mannheim. Ultrafiltration materials (amicon centripulus YM50) were obtained from Millipore Co.

2.2. Preparation of horse liver ADH

Horse liver ADH was dissolved in a 500 mM sodium phosphate buffer (pH 7.5) and freed of ethanol and NAD by gel filtration on a column of PD-10 with 10 mM sodium phosphate buffer (pH 7.5).

2.3. Affinity chromatography

A *p*-hydroxyacetophenone-Sepharose 6B column was prepared according to the method described by Ghenbot and

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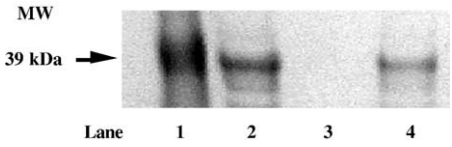

Fig. 1. Representative elution profile of horse liver alcohol dehydrogenase from a *p*-hydroxyacetophenone affinity column by acetylsalicylic acid. The polyacrylamide gel (12.5%) was stained with Commassie brilliant blue. Lane 1, molecular weight standard of yeast alcohol dehydrogenase (39 kDa), indicated by an arrow. Lane 2, commercial horse liver alcohol dehydrogenase. Lane 3, flow-through fraction from a *p*-hydroxyacetophenone affinity column. Lane 4, eluant from the column by acetylsalicylic acid (500 μM). Similar results were obtained from three different experiments.

Weiner (1992). Briefly, epoxy-activated Sepharose 6B was resuspended in deionized water (1 g/ml) and allowed to swell for 1 h at room temperature. The suspension was washed with 0.1 M NaOH solution and then resuspened in 0.1 M NaOH solution containing 0.5 M *p*-hydroxyacetophenone. After the pH had been adjusted to 11.0, the suspension was incubated at 45 °C for 15 h in a water shaker bath. The product was washed with ten volumes of deionized water. This was followed by successive washes with 0.1 M sodium bicarbonate buffer (pH 8.0) containing 0.5 M NaCl, with 0.1 M sodium acetate buffer (pH 4.0) containing 0.5 M NaCl, again with the first buffer, and then with deionized water. The product was placed in 100 mM ethanolamine (pH 8.0) and gently mixed overnight at room temperature. The gel was finally washed with deionized water, resuspended in 20 mM sodium phosphate buffer (pH 7.4) containing 50 mM NaCl, and stored at 4 °C in the presence of 0.02% sodium azide.

Prepared horse liver ADH was loaded onto a *p*-hydroxyacetophenone-Sepharose column that had been equilibrated with 20 mM phosphate buffer (pH 7.4) containing 0.1 mM dithiothreitol, 1 mM EDTA, 50 mM NaCl and 1 mM phenylmethanesulfonyl fluoride. The column was washed with a four column-volume of the equilibration buffer, and the proteins binding to the column were successively eluted with a two column-volume of the equilibration buffer containing 500 μM acetylsalicylic acid at a constant flow rate of 0.5 ml/min. Aliquots of the fraction eluted from the affinity column were pooled and then concentrated in an ultrafiltration cell. The concentrated samples were used for subsequent SDS-PAGE analysis.

2.4. Measurement of ADH activity using ethanol as a substrate

The enzymatic activity of horse liver ADH was measured in 0.1 M sodium phosphate buffer (pH 7.5), containing 2.5 mM NAD, 10 mM ethanol and 5 μg/ml of horse liver ADH at 25 °C, using a Beckmann DU7000 spectrophotometer. One unit of enzymatic activity (U) of alcohol equals 1 μmol of NAD(H) produced/min, based on an absorption coefficient of 6220/M/cm for NADH at 340 nm.

2.5. Protein determination

Protein concentration was measured by the Bradford method with bovine serum albumin as a standard.

2.6. Statistical analysis

The data are expressed as means ± SDs. One-way analysis of variance and multiple comparison using Scheffé *F*-test were used for statistical analysis. p values less than 0.05 were regarded as significant.

3. Results

3.1. Elution of horse liver ADH from a p-hydroxyacetophenone affinity column by acetylsalicylic acid

Fig. 1 shows elution profiles of horse liver ADH from a *p*-hydroxyacetophenone affinity column. *p*-Hydroxyacetophenone affinity matrix showed an ability to bind to ADH. Acetylsalicylic acid eluted ADH from the matrix. ADH was detected in the acetylsalicylic acid-eluted fraction but not in the flow-through fraction.

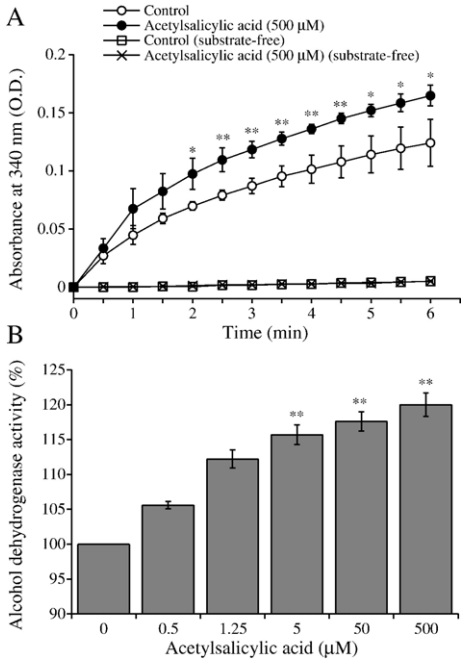

Fig. 2. (A) Time course of activation of horse liver alcohol dehydrogenase. The activation of ethanol oxidation was studied in the absence or presence of acetylsalicylic acid (500 μM) and in the absence or presence of ethanol (substrate, 10 mM) in 0.1 M sodium phosphate buffer (pH 7.5), containing 2.5 mM NAD and 5 μg/ml of horse liver alcohol dehydrogenase, at 25 °C. Asterisks denote significant difference from the control in the absence of acetylsalicylic acid (*$p<0.05$; **$p<0.01$). $n=3$. (B) Enhancement of horse liver alcohol dehydrogenase activity by different concentrations (5–500 μM) of acetylsalicylic acid. The activation of ethanol oxidation was studied in the absence or presence of acetylsalicylic acid (0.5, 1.25, 5, 50, 500 μM) in 0.1 M sodium phosphate buffer (pH 7.5), containing 2.5 mM NAD, 10 mM ethanol and 5 μg/ml of horse liver alcohol dehydrogenase, at 25 °C for 3 min. The data are expressed as the % of the control level in the absence of acetylsalicylic acid. Asterisks denote significant difference from the control in the absence of acetylsalicylic acid (**$p<0.01$). $n=3$.