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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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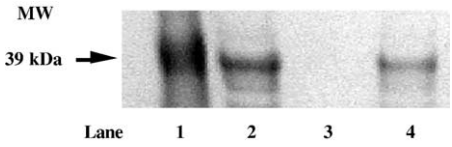

Fig. 1. Representative elution profile of horse liver alcohol dehydrogenase from a *p*-hydroxyacetophenone affinity column by acetylsalicylic acid. The polyacrylamide gel (12.5%) was stained with Commassie brilliant blue. Lane 1, molecular weight standard of yeast alcohol dehydrogenase (39 kDa), indicated 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.

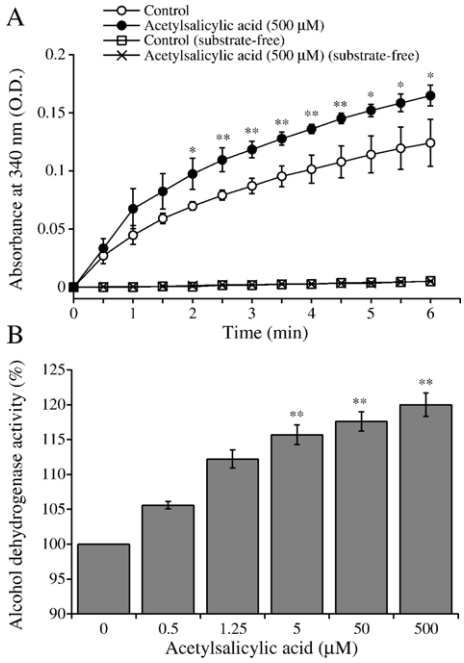

Fig. 2. (A) Time course of activation of horse liver alcohol dehydrogenase. The activation of ethanol oxidation was studied in the absence or presence of acetylsalicylic acid (500 μM) and in the absence or presence of ethanol (substrate, 10 mM) in 0.1 M sodium phosphate buffer (pH 7.5), containing 2.5 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 (**$p<0.01$). $n=3$.

3.2. Enhancement of enzymatic activity of horse liver ADH by acetylsalicylic acid

Effects of acetylsalicylic acid on ethanol oxidation by horse liver ADH were tested. In our preliminary experiments, acetylsalicylic acid up to 500 μM did not affect pH of the 0.1 M phosphate (pH 7.5)-containing assay buffer of ADH reaction and no NADH production in the assay solution was observed before addition of ethanol to the solution. However, acetylsalicylic acid at 625 μM or higher decreased the pH of the above phosphate buffer used for assay of ADH activity. Thus, acetylsalicylic acid at concentrations of 500 μM or below was used in the present study. The absorbance at 340 nm during the initial 6 min of the enzyme reaction in the presence of ethanol (10 mM) as a substrate was significantly increased by acetylsalicylic acid (500 μM) (Fig. 2A). Acetylsalicylic acid did not induce NAD-NADH reaction due to ADH in the absence of ethanol, a substrate of ADH (Fig. 2A). Thus, acetylsalicylic acid has no non-specific effect on ADH reaction. Acetylsalicylic acid (5–500 μM) significantly increased enzymatic activity of ADH in a concentration-dependent manner (Fig. 2B). Enzymatic activity of horse liver ADH was increased by acetylsalicylic acid (500 μM) from 4.5±0.8 (a vehicle-incubated control) to 5.4±1.0 U/mg (acetylsalicylic acid incubation). Both K_m and V_{max} were significantly increased by acetylsalicylic acid [K_m (mM), 1.38±0.09 (control) vs. 1.62±0.06 (acetylsalicylic acid incubation) ($p<0.05$); V_{max} (mol/min/mg), 8.77±0.29 (control) vs. 10.87±0.18 (acetylsalicylic acid incubation) ($p<0.05$)]. Thus, acetylsalicylic acid is a non-competitive enhancer of ADH activity.

4. Discussion

ADH plays crucial roles in the processes of detoxification of ethanol. We have recently demonstrated that liver cytosolic ADH binds to *p*-hydroxyacetophenone affinity matrix and that acetylsalicylic acid elutes ADH from the matrix (Negoro and Wakabayashi, 2004a,b). Using this character of ADH, we examined whether acetylsalicylic acid showed an affinity with commercial horse liver ADH. Acetylsalicylic acid also showed an ability to elute horse liver ADH from *p*-hydroxyacetophenone affinity chromatography, to which horse liver ADH had been applied. This result suggests that acetylsalicylic acid has a binding affinity to ADH and prompted us to investigate effects of acetylsalicylic acid on ADH activity. There have been only a few studies showing effects of acetylsalicylic acid on ADH activity and the blood alcohol concentration. Acetylsalicylic acid has been reported to decrease enzymatic activity of cytosolic ADH in human and rat gastric mucosa (Roine et al., 1990). However, much higher concentrations of acetylsalicylic acid were used in the above study than in the present study, and these high concentrations of acetylsalicylic acid might lower the pH of the assay solution (0.1 M glycine buffer), resulting in a decrease of ADH activity. In fact, our preliminary study demonstrated that acetylsalicylic acid at 625 μM or higher concentrations decreases ADH activity compared with that in the presence of 500 μM acetylsalicylic acid. Oral intake of 1 g of acetylsalicylic acid has been shown to significantly enhance the elevation of ethanol concentration in blood after ethanol consumption (Roine et al., 1990; Gentry et al., 1999), suggesting that acetylsalicylic acid inhibits elimination of ethanol from blood circulation. However, acetylsalicylic acid even at a high concentration (10 mM) did not affect ADH activity in the liver (Roine et al., 1990). On the other hand, administration of a low dose of acetylsalicylic acid (75 mg/day for one week) has been reported to decrease blood ethanol concentration (Kechagias et al., 1997). The reason for these inverse actions of acetylsalicylic acid on the blood concentration of ethanol is not known. There has been no report on direct interaction of acetylsalicylic acid with ADH in ethanol metabolism. The present study was therefore carried out to determine whether enzymatic activity of ADH in the presence of ethanol as a substrate is directly affected by acetylsalicylic acid, using horse liver ADH, which is often used for experiments as a kind of ADH of mammalian origin. Horse liver ADH activity was increased in the presence of acetylsalicylic acid. Both K_m and V_{max} were significantly increased by acetylsalicylic acid. Thus, acetylsalicylic acid is a non-competitive enhancer of ADH activity. We also examined the effects of acetylsalicylic acid on activity of commercial ADH from yeast, another source of ADH often used for experiments. Similarly to the results of the experiments using horse liver ADH, acetylsalicylic acid at 5–500 μM significantly enhanced activity of yeast ADH in a concentration-dependent manner [acetylsalicylic acid 5 μM, 106.8±1.1% of control ($p<0.05$); 50 μM, 114.3±3.2% ($p<0.01$); 500 μM, 117.3±3.8% ($p<0.01$)], although the degree of enhancement was not large. This is, to the best of our knowledge, the first study showing an enhancing effect of acetylsalicylic acid on enzymatic activity of ADH in vitro. Although the degree (20% at most) of enhancement of ADH activity by acetylsalicylic acid (5–500 μM) is not large, the concentrations of ADH used in the present study are clinically attainable (Rowland and Riegelman, 1968). In addition, the range of acetylsalicylic acid concentrations used was limited because acetylsalicylic acid at higher concentrations acidifies the assay solution for measurement of ADH activity as described above. In order to clarify the mechanism of the above action of acetylsalicylic acid on ADH, it is necessary to investigate conformational and biochemical interactions of acetylsalicylic acid with ADH. For this purpose, mutation of ADH directed to the binding site of acetylsalicylic acid would be useful.

Oral administration of acetylsalicylic acid and ethanol has been reported to decrease both the peak blood ethanol concentration (C_{max}) and the area under the blood ethanol curve (AUC), which are pharmacokinetic parameters in vivo (Kechagias et al., 1997). This finding agrees with the present finding that ethanol enhances enzymatic activity of ADH. The degree of enhancement of AUC by acetylsalicylic acid is comparable with the degree of enhancement of in vitro ADH activity by acetylsalicylic acid in the present study. However, the decrease in C_{max} and AUC of blood ethanol concentration was speculated in the above study to be due to delayed gastric emptying following ethanol ingestion. Further study on absorption of ethanol in gastric mucosa is needed to clarify the mechanism.

In conclusion, acetylsalicylic acid has a direct enhancing effect on enzymatic activity of ADH in vitro.

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