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INACTIVATION OF PULMONARY NAD$^+$-DEPENDENT 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE BY ACROLEIN

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Abstract—Acrolein, a highly reactive aldehyde found in cigarette smoke, was shown to induce time-dependent inactivation of NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase from porcine lung. The inactivation process followed pseudo-first-order kinetics and was irreversible. Inactivation by acrolein can be prevented by prior incubation of the enzyme with GSH but not by subsequent addition of GSH during assay. Inactivation can be also protected fully by prior incubation of the enzyme with NAD$^+$, but only partially with prostaglandin E_1. The results suggest that acrolein alkylates the enzyme at the coenzyme binding site and inactivates the enzyme. Inactivation of pulmonary NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase by acrolein may alter cellular and circulating thromboxane/prostacyclin ratios and subsequently disturb vascular homeostasis and augment inflammatory and anaphylactic responses in smokers.

Acrolein is a highly reactive α,β-unsaturated aldehyde found in cigarette smoke and automobile exhaust, and is a combustion product of fuels and cellulosic materials [1–3]. Its presence in products of combustion causes irritation of the respiratory system and may result in a variety of changes in the lung, including edema, hyperemia, altered cell migration, cytotoxicity and ciliostasis [4]. The mechanisms for these reactions in the acrolein-exposed lung are not well characterized.

Prostaglandins are a family of biologically potent fatty acids derived from arachidonic acid through the cyclooxygenase pathway [5]. Their roles in inflammation [6], in the immediate hypersensitivity response [7], and in numerous pulmonary diseases such as bronchial asthma, pulmonary edema, pulmonary thromboembolism and pulmonary distress syndrome [8, 9] have been strongly implicated. Prostaglandins are enzymically inactivated by oxidation to 15-keto derivatives catalyzed by NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) as they traverse the pulmonary circulation [10]. Therefore, inhibition or inactivation of pulmonary 15-PGDH may elevate cellular and circulating levels of prostaglandins. We have reported recently that pulmonary 15-PGDH activity is decreased by cigarette smoking [11]. We have suspected that acrolein, a poignant cigarette smoke component, may contribute in part to the decrease in enzyme activity since thiol reactive acrolein may inactivate sulfhydryl sensitive 15-PGDH [12]. In the work presented here, we show that acrolein can induce time-dependent inactivation of pulmonary 15-PGDH. Furthermore, we demonstrate that preincubation with NAD$^+$ but not prostaglandin E_1 (PGE_1) may protect the enzyme from inactivation, indicating that acrolein interacts with the enzyme at the coenzyme binding site.

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MATERIALS AND METHODS

Materials. Glutathione (GSH), DL-dithioreitol (DTT), NAD$^+$, *N*-ethylmaleimide, blue agarose, and Sephadex G-25 were purchased from the Sigma Chemical Co., St. Louis, MO. Hydroxyapatite (Bio-Gel HTP) was purchased from Bio-Rad Laboratories, Richmond, CA. Acrolein and propionaldehyde were supplied by the Aldrich Chemical Co., Milwaukee, WI. Prostaglandin E_1 was a gift of the Upjohn Co., Kalamazoo, MI. Porcine lung was obtained fresh from a local slaughter house and stored at −80°.

Enzyme assay. 15-PGDH activity was determined by following the formation of NADH fluorometrically as described previously [11]. The reaction mixture contained: 1.5 μmoles NAD$^+$, 15 nmoles PGE_1, and enzyme in a final volume of 1.5 ml of 0.05 M Tris–HCl buffer, pH 7.5. The reaction was initiated by the enzyme and allowed to proceed at room temperature. The rate of NADH formed was recorded by the increase in fluorescence at 468 nm with excitation at 340 nm, using an Aminco–Bowman SPF coupled to a recorder. The instrument was standardized by the known concentration of NADH determined by direct measurement of absorbance at 340 nm using $\varepsilon_M = 6.22 \times 10^3\ M^{-1}\ cm^{-1}$ [13].

Purification of 15-PGDH from porcine lung. The enzyme was purified to the step of DEAE-Sephadex A-50 according to a previously described procedure for human placenta [14]. Briefly, porcine lung (1 kg) was homogenized in 1 vol. of 0.01 M potassium phosphate buffer, pH 7.5, containing 1 mM DTT, 1 mM EDTA and 20% glycerol (Buffer A). The homogenate was centrifuged at 16,000 *g* for 45 min. The supernatant fraction was acidified to pH 6.1 with 1 N acetic acid and centrifuged at 105,000 *g* for 45 min. The supernatant fraction was applied to a blue agarose column (5.5 × 17 cm) equilibrated with Buffer

A. The column was washed with about 3 liters of the same buffer and then eluted with 20% glycerol in water containing 1 mM DTT and 0.1 mM NAD^+. The active fractions were pooled and applied into a hydroxyapatite cellulose column (3.5 × 15 cm). The column was washed with 0.02 M potassium phosphate buffer, pH 7.5, containing 1 mM DTT, 1 mM EDTA and 20% glycerol until $O.D._{280}$ of the effluent was less than 0.05. The enzyme was eluted with 0.05 M potassium phosphate buffer, pH 7.5, containing 1 mM DTT, 1 mM EDTA and 20% glycerol. The active fractions were pooled and concentrated by ultrafiltration using PM 10 membrane. The concentrated preparation was applied to a DEAE-Sephadex A-50 column (2.5 × 7 cm) equilibrated with Buffer A. The column was washed with 0.035 M potassium phosphate buffer, pH 7.5, containing 1 mM DTT, 1 mM EDTA, and 20% glycerol until the $O.D._{280}$ of the effluent was minimum. The enzyme was then eluted with 0.35 M potassium phosphate buffer, pH 7.5, containing 1 mM DTT, 1 mM EDTA and 20% glycerol. The active fractions were pooled and concentrated by ultrafiltration and stored at −80° in small aliquots. The specific activity of the purified enzyme was 117 mU/mg which represented over 400-fold purification.

Preparation of DTT and glycerol-free enzyme solution. DTT and glycerol were removed from the above purified concentrated enzyme solution (1 ml) by chromatography on a Sephadex G-25 column (1 × 18 cm) equilibrated with 0.05 M Tris–HCl buffer, pH 7.5. The void volume fraction which was devoid of DTT and glycerol was immediately used for inactivation studies.

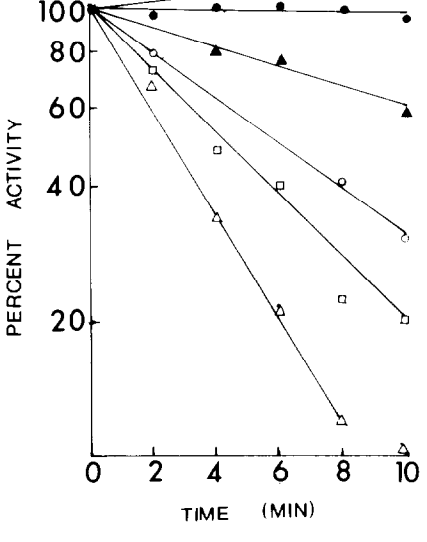

Fig. 1. Inactivation of pulmonary NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase by acrolein. Purified enzyme devoid of DTT was preincubated with different concentrations of acrolein or propionaldehyde for the indicated length of time before an aliquot was removed for enzyme assay. Key: (●) enzyme only; (■) 1 mM propionaldehyde; (▲) enzyme plus 5 μM acrolein; (○) enzyme plus 10 μM acrolein; (□) enzyme plus 20 μM acrolein; and (△) enzyme plus 50 μM acrolein.

RESULTS

When the native enzyme was incubated with acrolein, the loss of enzyme activity could be monitored as a function of time (Fig. 1). The inactivation rate increased as the concentration of acrolein increased.

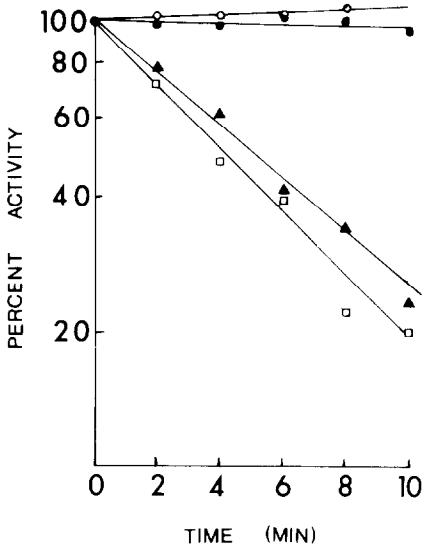

Fig. 2. Effect of GSH on acrolein-induced inactivation of pulmonary NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase. Purified enzyme devoid of DTT was preincubated with or without 1 mM GSH for the indicated length of time before an aliquot was removed for enzyme assay. Key: (●) enzyme only; (○) enzyme, 1 mM GSH and 20 μM acrolein during preincubation; (▲) enzyme plus 20 μM acrolein during preincubation and enzyme was then assayed in the presence of 1 mM GSH; and (□) enzyme plus 20 mM acrolein during preincubation.

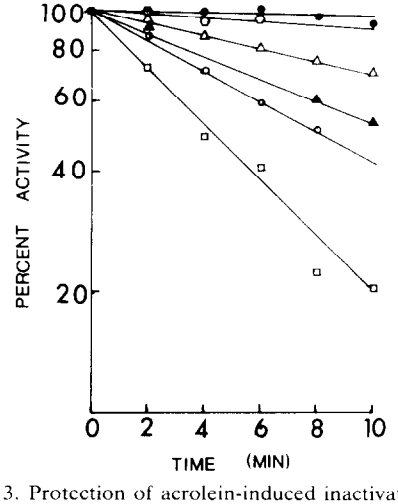

Fig. 3. Protection of acrolein-induced inactivation of pulmonary NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase by NAD^+. Purified enzyme devoid of DTT was incubated with 20 μM acrolein in the presence of different concentrations of NAD^+ for the indicated length of time before an aliquot was removed for enzyme assay. Key: (●) enzyme only; (□) enzyme plus 20 μM acrolein; (○) enzyme plus 20 μM acrolein and 20 μM NAD^+; (▲) enzyme plus 20 μM acrolein and 50 μM NAD^+; (△) enzyme plus 20 μM acrolein and 200 μM NAD^+; and (◌) enzyme plus 20 μM acrolein and 1 mM NAD^+.

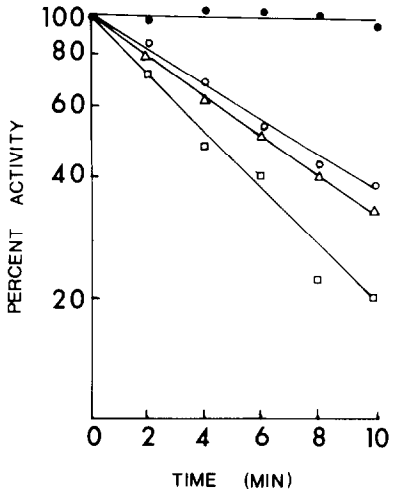

Fig. 4. Protection of acrolein-induced inactivation of pulmonary NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase by PGE_1. Purified enzyme devoid of DTT was incubated with 20 μM acrolein in the presence of different concentrations of PGE_1 for the indicated length of time before an aliquot was removed for enzyme assay. Key: (●) enzyme only; (□) enzyme plus 20 μM acrolein; (△) enzyme plus 20 μM acrolein and 70 μM PGE_1; and (○) enzyme plus 20 μM acrolein and 140 μM PGE_1.

and the reaction followed pseudo-first-order kinetics. However, under similar conditions, saturated propionaldehyde did not induce any enzyme inactivation. In fact, slight activation was observed. A replot of the inactivation half-life ($T_{\frac{1}{2}}$) as a function of the reciprocal of the acrolein concentration according to the method of Meloche [15] indicated a limiting half-life value of 1.3 min and a concentration of 50 μM for half-maximal rate of inactivation (K_i). Inactivation was prevented if acrolein was preincubated with 1 mM GSH before the addition of enzyme or if enzyme was preincubated with 1 mM GSH before the addition of acrolein. The inactivated enzyme could not be reactivated by incubation with 1 mM GSH or by assaying in the presence of 1 mM GSH (Fig. 2). When the enzyme was incubated with increasing concentrations of NAD^+, $T_{\frac{1}{2}}$ increased progressively and reached to nearly that of enzyme alone at 1 mM NAD^+ (Fig. 3). When the enzyme was incubated with the nearly saturated concentrations of PGE_1 ($K_m = 0.7$ μM), at a fixed level of acrolein, $T_{\frac{1}{2}}$ increased only slightly (Fig. 4).

DISCUSSION

The mechanism of inactivation of pulmonary 15-PGDH by acrolein is apparently related to the molecular reactivity of acrolein. Acrolein has two reactive groups, an α,β-carbon-carbon unsaturated bond and a carbonyl function. The presence of these two reactive groups suggests that acrolein could modify functional groups on a protein. Propionaldehyde is the saturated analog of acrolein with only one reactive site, the aldehyde group. This compound failed to inactivate 15-PGDH at 1 mM, suggesting that the α,β-unsaturated carbon-carbon bond is necessary for the observed activity of acrolein. Since 15-PGDH has been shown previously to be a sulfhydryl-sensitive enzyme [12], acrolein may well react with the sulfhydryl groups of the enzyme at the C-3 carbon. If the reaction of acrolein with the sulfhydryl group of the enzyme is necessary for the inactivation of enzyme, then the presence of excess sulfhydryl groups should protect the enzyme from inactivation by acrolein. Consistent with this hypothesis, reduced gluatathione did protect the enzyme from inactivation by acrolein. However, the enzyme could not be reactivated by GSH following enzyme inactivation by acrolein indicating an irreversible nature of inactivation. Although sulfhydryl groups of 15-PGDH are essential for activity, they may be important for maintaining the proper protein conformation instead of participating in the catalytic mechanism. To differentiate these two possibilities, inactivation of the enzyme by acrolein was carried out in the presence or absence of coenzyme NAD^+ or substrate PGE_1. Inactivation by acrolein appeared to be fully protected by 1 mM NAD^+ ($200 \times K_m$) but only slightly by 140 μM PGE_1, ($200 \times K_m$). Protection by NAD^+ is not due to possible reaction of NAD^+ with acrolein, since preincubation of acrolein with or without NAD^+ prior to the addition of enzyme gave the same reaction kinetics. These results suggest that acrolein alkylates sulhydryl residues at the coenzyme binding site.

Pulmonary NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase is one of the key enzymes controlling the levels of cellular and circulating biologically active and prostaglandins and thromboxanes. It has been reported that prostaglandins are rapidly inactivated as they traverse the pulmonary circulation [10] but prostacyclin passes through unchanged [16]. Thromboxane A_2 (TXA_2) may also be inactivated by pulmonary oxidation to 15-keto-TXA_2 followed by hydrolysis to 15-keto-TXB_2 in addition to inactivation by direct hydrolysis to TXB_2. This contention is supported by the findings that 15-keto-TXB_2 and TXB_2 are two major metabolites of arachidonate in perfusate of challenged sensitized guinea pig lung [17] and that 15-keto-TXB_2 is not likely to derive from TXB_2 since TXB_2 is not a substrate for 15-PGDH [12]. Therefore, inactivation of pulmonary 15-PGDH by acrolein may increase circulating thromboxane/prostacyclin ratios. Consequently, vascular homeostasis is disturbed, favoring aggregation of platelets and subsequent thrombus formation. Furthermore, impaired catabolism of pulmonary prostaglandins induced by acrolein may result in elevated prostaglandin and thromboxane levels which may augment inflammatory and anaphylactic responses. Recently, Grundfest *et al.* [18] reported that acrolein could also decrease PGE_2 synthesis without affecting TXB_2 synthesis in rat pulmonary alveolar macrophages presumably by inactivating prostaglandin endoperoxide E isomerase, a sulfhydryl sensitive enzyme. Consequently, vasoconstrictive and proaggregatory TXA_2 prevails in the pulmonary system following acrolein inhalation. Acrolein can modify synthesis as well as catabolism of prostaglandins and thromboxanes in the lung.

Inactivation of dehydrogenases by acrolein was

reported previously by Rando [19]. He demonstrated that acrolein inactivates yeast alcohol dehydrogenase and that NAD^+ protects the enzyme. However, inactivation occurred at a much higher concentration (mM range) of acrolein and at a much slower rate. Apparently, pulmonary 15-PGDH is very sensitive to acrolein inactivation. It is also conceivable that acrolein may inactivate other sulfhydryl sensitive enzymes. Therefore, smoking may not only affect arachidonate metabolism and vascular homeostasis but also alter other cellular functions mediated by sulfhydryl sensitive biomolecules.

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