

Complexity of Enzymatic Inhibition by Cigarette Smoke

One suggested mechanism for the tumorigenic effect of cigarette smoke in animals is enzymatic inhibition¹. Most published studies indicate that inhibition of sulfhydryl-containing enzymes is the mode of action¹⁻⁴, although other mechanisms may be operative⁵. Peroxides², acetaldehyde¹, free radicals⁴ and hydrogen cyanide⁵ have been implicated as the inhibitors in smoke. All these studies appear to suffer from the premise that a single compound or class of compounds in smoke is responsible for the inhibition. However, the occurrence of many non-competitive enzyme inhibitors in smoke is well known, e.g. hydrogen sulfide, carbon monoxide and phenol⁶. A priori, a complex pattern of inhibition would be expected, and the present report provides data on this point.

Smoke components may occur in the vapor phase (VP), the particulate matter phase (PM), or both phases⁶. By passing smoke from a cigarette through a special spun glass filter (Cambridge pad), the particulate matter may be removed preferentially, and the inhibiting activity of each phase may be examined independently. Also, by using a charcoal filter on a cigarette, some components in VP are selectively removed, thus providing a further means of studying relationships between smoke composition and inhibition.

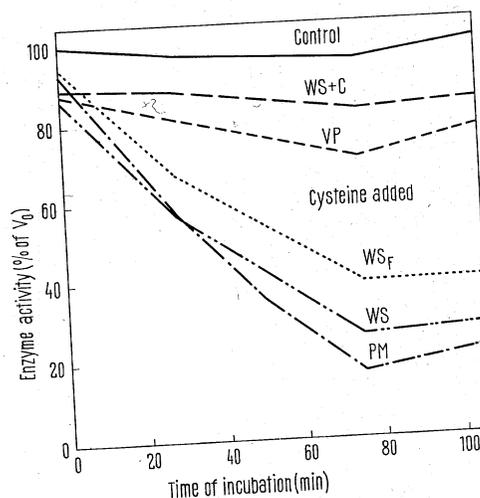
In the present work, 85 mm commercial, filter (combined charcoal and cellulose acetate) cigarettes were smoked with or without the filter under standard conditions⁷ and whole smoke (WS) or VP of smoke was collected by bubbling through buffer (1 cigarette/5 ml of 0.067 M phosphate buffer, pH 7.6). PM was obtained by extracting the material on Cambridge pads (used when VP was collected) with buffer to give the equivalent of 1 cigarette/5 ml. The inhibitory properties of filtered and unfiltered WS and separated phases thereof for yeast alcohol dehydrogenase are shown in the Figure. According to recent interpretations of the kinetics of enzymatic inhibition⁸, the initial inhibition (zero time) in the Figure can be considered a reflection of competitive and non-competitive equilibrium effects. Subsequent inhibition may be due to several types of enzyme-inhibitor or enzyme-inhibitor-substrate reactions which may be collectively termed 'inactivation'⁸. Unfiltered WS shows strong inactivation and PM contributes more to this pattern than VP. Inactivation by PM is greater than WS, indicating that VP and PM do not act additively. This pattern was confirmed by the inhibition of 'reconstituted smoke', prepared by mixing separated VP and PM (not shown), which gave an inactivation curve crudely approximating WS and less inhibitory than PM alone. The addition of cysteine after 75 min distinctly reverses the inactivation by both phases of smoke, but the effect is not so readily apparent with WS. However, concurrent exposure of cysteine and WS to the enzyme shows that the amino acid protects the enzyme effectively and reduces greatly the rate of enzymatic inactivation. These data confirm in part earlier findings^{2,3}. As expected, filtered WS gives less inhibition than unfiltered WS.

Removal of the charcoal from the filter results in a distinct increase in the initial inhibitory effect compared with smoke passed through the intact filter. In representative data using a substrate concentration of about $3.5 K_m$ ⁹, initial inhibitory velocities (% of control) by WS were 87 (no filter), 95 (filter intact) and 91 (filter with charcoal removed). Concurrent gas chromatographic analysis indicated that the charcoal filter reduces many of the volatile smoke components, including a 70% decrease in acetaldehyde¹⁰; this compound is found only in

VP¹¹ and would be expected to act as a competitive inhibitor.

Although determinations of inhibition type on complex mixtures by classical reciprocal plots of substrate concentration versus velocity may be tenuous^{8,9}, LINEWEAVER-BURK¹² and DIXON¹² plots were examined to obtain further information. Since concentrations of unidentified smoke inhibitors were obviously unavailable, complete kinetic analyses could not be made. However, the results reflect the complexity of inhibition by the smoke solutions. In general, VP appeared to be almost completely competitive; PM and WS showed neither classical competitive nor non-competitive inhibition but gave an apparent mixed inhibition.

PM contains many compounds which can act non-competitively, including phenols, nicotine, pyridine,



Yeast alcohol dehydrogenase inhibition by cigarette smoke and phases thereof. Smoke solution was mixed with an equal vol. of enzyme solution (10 μ g/ml buffer) and incubated at 25°C for the times shown. Aliquots (1.0 ml) were removed and added to the reaction mixture (200 μ moles ethanol, 300 μ moles NAD, 50 μ moles pyrophosphate buffer, pH 8.8; total vol. 2.0 ml), and the rate of NADH formation was read at 340 nm. V_0 , initial velocity (control); VP, vapor phase, non-filtered; PM, particulate matter phase, non-filtered; WS, whole smoke, non-filtered; WS_F, whole smoke, passed through charcoal-acetate type filter; WS + C, whole smoke, non-filtered, with cysteine (0.1 mole) added at zero time.

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alkylating agents, and free radicals⁶. Yeast alcohol dehydrogenase is inhibited competitively by pyridine¹³, substituted 'pyridinium ring' compounds¹⁴, and the alkylating agent, chloroquine¹⁵. Phenol and substituted phenols may act non-competitively, but also have been shown to compete with NAD in certain dehydrogenase systems¹⁶. Alcohol dehydrogenase is inactivated by X-radiation¹⁷ presumably by radical formation, and cysteine has a limited protective effect¹⁸. In model experiments, DIXON plots indicated that nicotine, and to some extent, phenol, gave competitive inhibition in the above enzyme system, whereas pyridine at the usual smoke concentration had a negligible effect. Inactivation by nicotine (PM component), pyridine (PM and VP component), hydrogen cyanide or carbon monoxide (VP components) does not seem to account for the observed inhibition by the smoke phases.

The occurrence of small amounts (1–2 μg /cigarette) of peroxides in smoke has been claimed¹⁹, but their presence could not be confirmed by enzymatic analysis in the present work. Hydrogen peroxide (30 μg) inhibited both initially and subsequently, but did not account for the overall inhibition. Ultracentrifugal analysis and acrylamide gel electrophoresis of the smoke-reacted enzyme revealed no evidence for the presence of dimers which are known to form by reactions between weak oxidants and sulfhydryl groups of proteins²⁰. However, disulfides may also be formed intramolecularly rather than by dimerization. Strong oxidants may also react with sulfhydryls, yielding sulfinic, sulfonic and related acids which are not reduced by addition of cysteine. The presence in cigarette smoke of both types of oxidants is probable from the analysis of the various effects of cysteine.

Zusammenfassung. Die Hemmung der Alkoholdehydrogenase aus Hefe durch Zigarettenrauch beruht auf verschiedenen Inaktivierungsmechanismen. Die Gasphase zeigt eine kompetitive Hemmung, die Partikelphase und der ganze Rauch eine gemischte Hemmung. Da die Hemmung durch den ganzen Rauch nicht der Summe der Hemmungen beider Phasen entspricht, ist anzunehmen, dass Wechselwirkungen zwischen den Inhibitoren und Antagonisten vorliegen.

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