ALTERED SUSCEPTIBILITY OF COLLAGEN TO COLLAGENASE DIGESTION AS A CONSEQUENCE OF EXPOSURE TO TOBACCO SMOKE

W. S. Rickert,
Department of Statistics,
University of Waterloo,
Waterloo, Ontario, Canada.

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SUMMARY

Exposure of calf skin acid soluble collagen to tobacco smoke leads to a dose-dependant decrease in solubility and lysine and hydroxlysine content, together with a concomitant decrease in susceptibility to collagenase digestion. Moreover, an approximately linear relationship was observed between the logarithm of the half-time required for digestion of the smoke-exposed collagen samples and the percentage of lysine residues modified. No significant loss in susceptibility to collagenase digestion was detected in collagen preparations when most of the lysine residues were converted to homocitrulline prior to exposure.

INTRODUCTION

During collagen maturation lysine and hydroxylysine residues undergo a number of modifications (1-3). Additional post-maturation modifications have been postulated to explain the age-related changes in macrorheological properties (4), reactivity of lysine residues (5-7), and in susceptibility to digestion by collagenase (8,9). Some of these changes can be simulated by *in vitro* chemical modification of lysine residues in 'young' or soluble collagen preparations (4,10-12), but generally, it has not been possible to demonstrate similar alterations *in vivo* (13).

Recently it has also been shown that exposure of certain collagen preparations to the gaseous phase of tobacco smoke results in a dose-dependant loss in solubility and decrease in lysine content (14). In addition, there is some suggestive evidence that modification of collagen may also occur *in vivo* from this type of environmental exposure (15-17). The present communication demonstrates that susceptibility to digestion by collagenase can be used to evaluate the effects of tobacco smoke on collagen.

MATERIALS AND METHODS

Acid soluble collagen (ASC) was a product of Calbiochem, California and chromatographically purified collagenase free of peptidase and trypsin-like activity was obtained from the Sigma Chemical Company. Both ASC and collagenase were used without further purification.

Carbamyl-ASC was prepared essentially according to the procedure of Stark and Smyth (18). Solid KCNO was added to a 0.1% solution of ASC at 50^{o} in 8 M urea and N-ethylmorpholine acetate buffer (pH 8.0). The temperature was maintained within $\pm$ 0.1^{o} C during the reaction period and the pH was kept constant by the periodic addition of 3M HCl (19). After 24 hr, the carbamylated protein was separated from excess reagent by extensive dialysis against distilled water, and lyophilyzed. The extent of carbamylation was established by amino acid analysis, with appropriate corrections being applied for the decomposition of homocitrulline (19).

Smoke-exposed samples of ASC were prepared under standard conditions, and lysine contents established by amino acid analysis (14). The digestion of ASC, carbamyl-ASC and smoke-exposed ASC was carried out as described previously (20).

RESULTS AND DISCUSSION

Although the conditions chosen for the carbamylation of ASC,i.e. 8M urea at a temperature of 50^{o} for 24 hours, have been considered sufficient for the denaturation of most proteins (18), conversion of all the lysine residues present to homocitrulline could not be accomplished. An average value of 7 unreacted lysine residues after 24 hours (table 1) is similar to the value of 6.4 reported earlier for calf skin tropocollagen (5) but greater than a more recent estimate of 2.1 lysine residues (7).

Table 1 and figure 1 show that exposure of ASC to tobacco smoke leads to a progressive decrease in susceptibility to collagenase digestion, as measured by the half-time required for complete digestion. This decrease is ascribed to a progressive modification of ASC and not of the enzyme since all preparations both modified and 'native', afforded a similar same end point after 5hr.

TABLE 1

LYSINE CONTENTS AND DIGESTION HALF-TIMES ($t_{\frac{1}{2}}$) OF SMOKE-EXPOSED AND CARBAMYLATED SAMPLES OF CALF SKIN ACID SOLUBLE COLLAGEN

EXPOSURE + (NO. OF CIGARETTES)	LYSINE CONTENT ∇ (RESIDUES/ 10^5g)	DIGESTION HALF-TIME° (MIN)	TOTAL KOH CONSUMPTION * (μeq)
Controls	26.7	3.1	19.60
2	26.0	4.2	19.94
4	23.5	7.6	19.80
10	22.0	12.2	19.65
20	20.8	16.6	19.82
35	17.4	58.2	19.10
Carbamylated (35 cigarettes)	6.7	7.8	19.50
Carbamylated (No Exposure)	7.0	3.5	20.80

+ ASC and Carbamyl-ASC were exposed to the gaseous phase of tobacco smoke as described previously (14).

∇ Average of three values from amino acid analysis (14). Except for a decrease in hydroxylysine content (14), no significant changes in amino acid content were detected.

° Time required to consume one-half of the total amount of alkali (average of two values).

* Amount of KOH consumed by 30ml of a 0.1% solution after 5 hours digestion (20). Values are corrected for alkali uptake by a solution of protein without added enzyme.

Next, susceptibility to collagenase digestion appears to be related to the number of lysine residues modified in the collagen samples. Specifically, when the logarithm of the half-times required for the complete digestion of smoke-exposed samples was plotted against the percentage of modified lysine residues, an approximately linear relationship was obtained (figure 2). A similar relationship has previously been noted, (9), when the logarithm of the half-times required for the digestion of prepartions of insoluble collagen from human diaphragm tendon was plotted against age. However, this age-dependent loss in collagenase susceptibility may be simply a reflection of progressive post-maturation changes in the state of some of the lysine

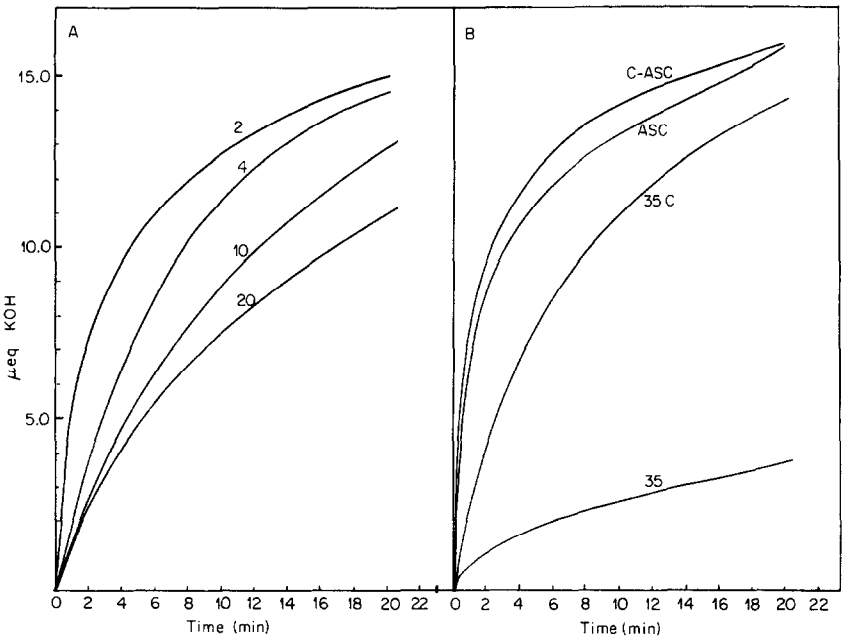

FIGURE 1: Initial rates of KOH consumption, at pH 7.6 and 37°, 30ml of a 0.1% suspension of collagen containing 300μg of collagenase (20). The numbers indicate the degree of exposure to cigarette smoke (number of cigarettes). In figure 1B, C-ASC: Carbamyl-ASC, no exposure; ASC: Acid soluble collagen, no exposure; 35C: Carbamyl-ASC exposed to 35 cigarettes; and 35: ASC exposed to 35 cigarettes.

residues since it is known that the number of unreactive lysine residues increases with age (5-7). Nevertheless, since both aging and exposure to cigarette smoke appear to modify the susceptibility to collagenase digestion and the lysine content of collagen samples, the results suggests the possibility that, in this regard at least, exposure to tobacco smoke mimics the effect of normal aging.

On the other hand modifications of lysine do not necessarily lead to a decreased collagenase susceptibility. For example, conversion of more than 70% of the lysine residues to homocitrulline had little effect on the half-time required for digestion. Also, once the majority of the lysine residues had been modified in this way, maximum exposure to cigarette smoke (35 cigarettes) resulted in only a small reduction in half-times (see figure 1B). Evidently

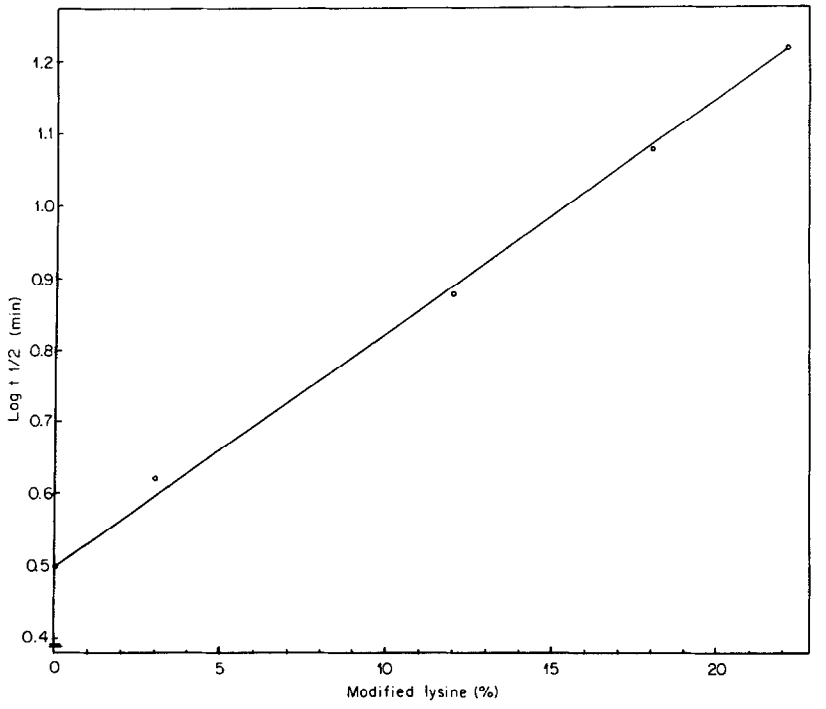

FIGURE 2: Logarithms of the time required for consumption of one-half of the total amount of KOH as a function of the percentage of the number of modified lysine residues (see table 1 for details).

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then, exposure of collagen to cigarette smoke, brings about a specific type of modification necessary before a reduction in susceptibility to collagenase digestion occurs.

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