Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 2041–2045, March 1982
Medical Sciences

Potential mechanism of emphysema: α_1-Proteinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase inhibitory capacity

(oxidant/obstructive lung disease/leukocyte)

HARVEY CARP*, FREDERICK MILLER*, JOHN R. HOIDAL†, AND AARON JANOFF*‡

*Department of Pathology, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York 11794; and †Department of Internal Medicine, University of Minnesota Health Sciences Center, Minneapolis, Minnesota 55455

Communicated by Bentley Glass, November 23, 1981

ABSTRACT **The elastase inhibitory capacity per mg of α_1-proteinase inhibitor (α_1PI) was measured in the bronchoalveolar lavage (BAL) fluid from 26 healthy smokers and 24 nonsmokers. Activity was decreased by 40% in smokers' BAL fluid compared to nonsmokers. This effect was demonstrable by using human neutrophil elastase as well as porcine pancreatic elastase as test enzyme (elastase, EC 3.4.21.11) and was reproducible when selected individuals in each group underwent lavage on repeated occasions. In contrast, the functional activity of α_1-antichymotrypsin was not decreased in smokers' BAL fluid. Crossed antigen–antibody electrophoresis confirmed that inactivation of α_1PI was responsible for the decrease in the elastase inhibitory capacity of smokers' BAL fluid. α_1PI purified from smokers' BAL fluids contained methionine sulfoxide (4 mol/mol of inactive α_1PI), whereas α_1PI from nonsmokers' BAL fluid did not. Smokers' α_1PI was indistinguishable from nonsmokers' α_1PI on the basis of electrophoretic mobility, molecular weight, and immunoreactivity. Thus, oxidation of methionine residues in lung α_1PI is associated with cigarette smoking. Because chemical oxidation of α_1PI *in vitro* causes loss of its elastase inhibitory activity, the present observations suggest that methionine oxidation may also be the cause of decreased functional activity of lung α_1PI in smokers. Oxidative inactivation of α_1PI in the lungs of cigarette smokers may play a role in the development of pulmonary emphysema in this group.**

α_1-Proteinase inhibitor (α_1PI, α_1-antitrypsin) is a major regulator of polymorphonuclear neutrophil elastase (EC 3.4.21.11) in the lower respiratory tract of man (1). Severe genetic deficiency in α_1PI is frequently associated with the development of pulmonary emphysema (2). This association, plus the production of emphysema in experimental animals by treatment with intrapulmonary elastase (3), has led to the hypothesis that pulmonary emphysema may be caused by an imbalance between elastases and their naturally occurring inhibitors in the lung tissue (4). Because the majority of emphysema patients have normal levels of α_1PI in their circulation and because a major risk factor in their disease appears to be cigarette smoking (5), attention has recently been focused on potential disturbances of elastase–α_1PI balance in the lung by inhaled tobacco smoke.

Earlier, we and others showed that cigarette smoke inactivated α_1PI *in vitro* (6–8). In addition, a significant loss of α_1PI activity has been documented in bronchoalveolar lavage (BAL) fluids obtained from rats immediately after acute inhalation exposure to cigarette smoke (9) and obtained from human chronic smokers (10).

Several lines of evidence suggest that α_1PI inactivation by cigarette smoke may be due to oxidation of the active site methionine residue in the inhibitor (11, 12). For example, treatment of human α_1PI with chemical oxidants (7, 12–14) causes a decrease in its capacity to inhibit elastase and a parallel formation of methionine sulfoxide in the inhibitor (12–14). Furthermore, α_1PI will retain normal elastase inhibitory capacity (EIC) even in the presence of smoke solution, if free radical scavengers are first added to the smoke extract (7). Moreover, the decreased activity of α_1PI recovered from BAL of smoke-exposed rats or oxidant-treated serum can be partially restored by subsequent treatment of the inhibitor with a reducing agent (8, 9). Incubation of α_1PI *in vitro* with purified myeloperoxidase or stimulated phagocytes (another potential source of oxidant in the lung, in addition to cigarette smoke) results in the oxidative inactivation of the inhibitor (15–17). Finally, inactive α_1PI purified from inflammatory synovial fluid (where the inhibitor is exposed to oxygen free radicals and peroxidative enzymes secreted by inflammatory leukocytes) has been shown to contain methionine sulfoxide residues (18).

The purpose of the present study was to test directly whether methionine sulfoxide residues could be demonstrated in α_1PI recovered from the lungs of cigarette smokers. Although such a finding, by itself, does not prove that oxidation of α_1PI by cigarette smoke causes pulmonary emphysema, our results at least confirm the presence of inactive α_1PI in smokers' BAL fluid and demonstrate significant methionine sulfoxide content in the inhibitor protein.

MATERIALS AND METHODS

BAL. BAL fluid was obtained from normal smoking and nonsmoking volunteers through a fiberoptic bronchoscope by standard technique (19). Smokers were permitted to smoke up to the time of the lavage procedure. Informed consent was obtained from all of the subjects prior to bronchoscopy and lavage. Prior to analysis, an aliquot of frozen BAL cell-free supernate was concentrated approximately 50-fold by negative pressure membrane ultrafiltration (Immersible CX-10, Millipore) or by oncotic methods (Aquacide II-A, Calbiochem) and used directly to measure α_1PI functional activity.

Analysis of BAL Fluid. Albumin, α_1PI, and α_1-antichymotrypsin (α_1Achy) were all measured by radial immunodiffusion.

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Abbreviations: α_1Achy, α_1-antichymotrypsin; α_1PI, α_1-proteinase inhibitor; BAL, bronchoalveolar lavage; CIC, chymotrypsin inhibitory capacity; EIC, elastase inhibitory capacity; PPE, porcine pancreatic elastase.

‡To whom reprint requests should be addressed.

EIC was measured by using porcine pancreatic elastase (PPE) (Elastin Products, St. Louis, MO) or human neutrophil elastase purified by us (20) as test enzyme with succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide (Peninsula Laboratories, San Carlos, CA) as substrate (21). Chymotrypsin inhibitory capacity (CIC) was measured by using bovine pancreatic chymotrypsin (Worthington) as test enzyme and *N*-benzoyltyrosine thiobenzyl ester (a gift of E. Shaw, Brookhaven National Laboratory, Upton, NY) as substrate (22). Complexes of α_1PI with elastase or free elastase were demonstrated by crossed antigen–antibody electrophoresis (23), using antibodies against human α_1PI (Accurate Scientific, Westbury, NY) and antiserum against PPE (prepared by us).

Purification of α_1PI. Approximately 18 liters of BAL fluid from 26 smokers and 24 nonsmokers listed in Table 1 were combined into two pools, concentrated as before, dialyzed against 0.05 M ammonium bicarbonate (pH 7.8), lyophilized, and used for purification of α_1PI by immunoaffinity chromatography (24). The run-through fractions proved to be free of α_1PI and were used to measure the functional activity of α_1Achy in the BAL fluids. α_1PI was eluted from the washed column by applying 6 bed volumes of buffer containing 6 M guanidine hydrochloride. The eluted inhibitor was then dialyzed against 0.05 M ammonium bicarbonate (pH 7.8) and lyophilized prior to further analysis. In some experiments, human serum or *N*-chlorosuccinimide-treated (oxidized) serum (7) was used as starting material in place of concentrated BAL fluid. Recovery of α_1PI from BAL fluid or serum varied between 25% and 45%. In all cases, purified α_1PI migrated as a single band in $NaDodSO_4$/polyacrylamide gel electrophoresis and gave a single precipitin peak when analyzed by crossed antigen–antibody electrophoresis against antibodies to whole human serum (Kalestad Laboratories, Chaska, MN).

Amino Acid Analysis of α_1PI. The methionine sulfoxide content of α_1PI was measured by the procedure of Shechter *et al.* (25). Purified α_1PI (10 nmol) was treated for 24 hr with a 100-fold excess of cyanogen bromide in 70% (vol/vol) formic acid. This step converts free unoxidized methionine to homoserine and homoserine lactone, without affecting oxidized methionine residues. Cyanogen bromide was then removed by lyophilization and the samples were hydrolyzed for 24 hr at 100°C with redistilled, 5.6 M (constant-boiling) HCl under reduced pressure. Dithioerythritol (1.0 mg) was also present during hydrolysis in order to reduce methionine sulfoxide back to methionine. All samples were hydrolyzed and analyzed in triplicate. As recommended by Shechter *et al.* (25), the value obtained for methionine was taken as the amount of methionine sulfoxide originally present in the protein. However, this value was first corrected for any methionine residues that had not been attacked by cyanogen bromide. This correction factor was always <5% of total methionine. A standard preparation of *N*-chlorosuccinimide-treated (oxidized) α_1PI (12) was also analyzed as a positive control. Other amino acids (except tryptophan) were measured in the same hydrolysate used for methionine analysis. Tryptophan in α_1PI was measured by fluorescence photometry in the presence of 6 M neutralized guanidine hydrochloride (290-nm excitation, 340-nm emission).

Molecular Weight Determination. $NaDodSO_4$ polyacrylamide gel electrophoresis was performed by a modification of the technique of Weber and Osborn (26), in which $NaDodSO_4$ was present only in the sample solution and not in the electrophoresis or gel buffer (0.1 M phosphate buffer, pH 8.0) (27). After electrophoresis, the $NaDodSO_4$-treated α_1PI was identified immunologically as described (28).

Thin-layer gel immunofiltration analysis of α_1PI was performed as described (29), using Sephadex G-150 (Pharmacia).

RESULTS

Functional Activity of α_1PI in BAL Fluid. A preliminary experiment was carried out to test the assumption that the EIC of BAL fluid is due to α_1PI. Specific antibodies directed against α_1PI or control immunoglobulin (both free of endogenous elastase inhibitors) was incubated with BAL fluids for 15 min at 37°C, after which the remaining EIC was measured. Treatment with anti-α_1PI eliminated the EIC of the BAL fluids (when either PPE or neutrophil elastase was used as test enzyme), whereas control immunoglobulin was without effect. Thus EIC of BAL fluid is primarily due to the anti-elastase activity of α_1PI, as has also been suggested by others (1).

Experiments were next undertaken to evaluate the EIC per unit of BAL fluid α_1PI (measured immunologically) in smokers and nonsmokers. As shown in Table 1, α_1PI activity was decreased in BAL fluid of smokers in comparison to nonsmokers, and the effect was not related to subject age or sex. Both groups had similar levels of antigenic α_1PI in their BAL fluid (nonsmoker α_1PI/albumin = 57 ± 12 μg/mg, smoker α_1PI/albumin = 65 ± 20 μg/mg, mean of three measurements ± 1 SD). Within the smoking group, the degree of reduction in functional α_1PI did not appear to be correlated with the total number of pack-years of smoking or the total number (or type) of cells recovered in BAL fluid (data not shown).

The data in Table 1 suggest that smokers' lung α_1PI is, on average, only 59% as effective an anti-elastase as is α_1PI in nonsmokers' BAL fluid. These data confirm the previous results of Gadek *et al.* (10). The experiments in Table 1 utilized BAL fluids that had been concentrated prior to EIC assay, as described in *Materials and Methods*. However, similar results were obtained with unconcentrated pooled BAL fluids (data not shown), ruling out the possibility that α_1PI inactivation by smoke components occurred secondarily during concentration of the fluids.

The experiments outlined in Table 1 utilized PPE to test the anti-elastase activity of BAL fluid α_1PI. In addition, separate pools of smokers' and nonsmokers' BAL were analyzed, using

Table 1. Functional activity of α_1PI in smokers' and nonsmokers' BAL fluid

Category	No.	Male	Female	Age,* years	Smoking history, pack-years*	EIC per μg α_1PI†
Nonsmokers	24	11	13	25 ± 6.8	0	0.59 ± 0.08‡
Smokers	26	17	9	28 ± 3.2	18.1 ± 3.5	0.34 ± 0.10§

* Mean ± 1 SD. A pack-year is 1 pack per day for a year.

† Micrograms of PPE inhibited after incubation for 10 min at 37°C per μg of α_1PI (measured immunologically) in concentrated BAL fluid, mean of at least three measurements ± 1 SD.

‡ The control value of 0.59 μg of elastase inhibited per μg of α_1PI exceeds the theoretically calculated ratio of 0.50, based on 1:1 molar interaction between enzyme (M_r 26,000) and inhibitor (M_r 52,000). The discrepancy resulted from impurities in the commercial elastase preparation used in our experiments.

§ Significantly different from nonsmokers' value, $P < 0.05$ (t test).

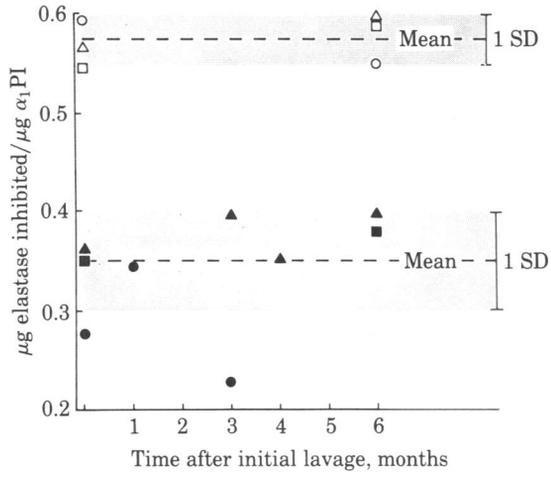

FIG. 1. Persistence of decreased functional activity of α_1PI in smokers' BAL fluids. Three smoking subjects (●, ■, ▲) and three nonsmoking subjects (○, □, △) underwent repeated BAL over a period of 6 months. PPE was used as test enzyme, and all other conditions were identical to those described in Table 1. For the three smokers, α_1PI functional activity was depressed at the time of the initial measurement and remained depressed over a period of 3–6 months.

human neutrophil elastase as test enzyme. After a 15-sec incubation with neutrophil elastase at 37°C, smokers' BAL fluid showed only 60 ± 10% of the inhibitory activity found in nonsmokers' BAL fluid when expressed per μg of α_1PI, measured immunologically (mean of three experiments ± 1 SD). However, after a 30-min incubation with neutrophil elastase at 37°C, smokers' BAL fluid showed 88 ± 12% of the inhibitory activity of nonsmokers' BAL fluid per μg of α_1PI (mean of three experiments ± 1 SD). By contrast, incubation of smokers' BAL fluid with PPE for as long as 40 min did not result in significant recovery of inhibitory activity (data not shown). Similar differences in the time course of inhibition of neutrophil and pancreatic elastase have been reported for chemically oxidized α_1PI *in vitro* (13). Although oxidized α_1PI slowly inhibits human neutrophil elastase *in vitro*, such a decreased rate of inhibition increases the likelihood that enzyme released under conditions *in vivo* could more readily reach and damage connective tissue components.

We next evaluated functional activity of α_1PI in the BAL fluid of several individual smokers and nonsmokers who underwent repeated lavage over a 6-month period. As shown in Fig. 1, individual smokers repeatedly showed decreased lung α_1PI functional activity compared to nonsmokers.

We also measured the functional activity of α_1Achy in BAL fluids from which α_1PI had first been removed by immunoabsorption (see *Materials and Methods*). First, α_1Achy was found to be responsible for essentially all of the CIC of α_1PI-depleted BAL fluid, because specific antibodies directed against α_1Achy (free of endogenous chymotrypsin inhibitors) eliminated the CIC of such fluids. Second, the CIC per unit of BAL fluid α_1Achy (measured immunologically) in smokers' BAL fluid was 90 ± 10% of the CIC of nonsmokers' BAL fluid ($P > 0.2$, mean of three measurements ± 1 SD). These results suggest that nonspecific effects of cigarette smoke on lung proteins were probably not responsible for the decreased activity of α_1PI observed in smokers' BAL fluid.

Analysis of BAL Fluids by Crossed Antigen–Antibody Electrophoresis. To confirm that the decrease in smokers' BAL fluid EIC was due to inactivation of α_1PI, separately pooled samples of smokers' and nonsmokers' BAL fluids were incubated with PPE as before and then analyzed by crossed antigen–antibody electrophoresis. As shown in Fig. 2, α_1PI in nonsmokers' BAL fluid was able to complex all of the added PPE, whereas the

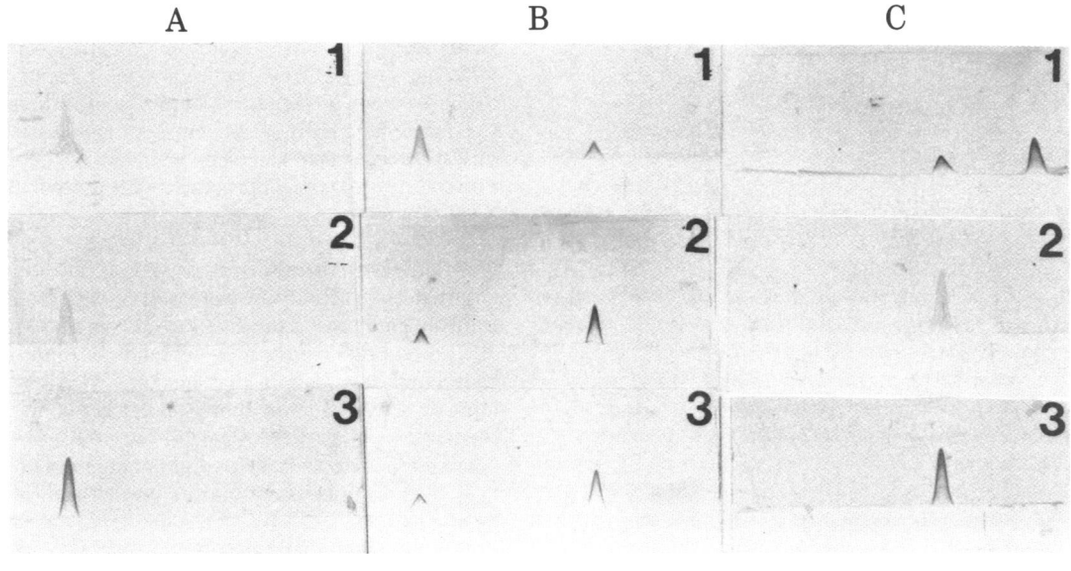

FIG. 2. Crossed antigen–antibody electrophoretic analysis of mixtures of elastase and BAL fluid or serum. The gels in *A* and *B* contain 2% (vol/vol) anti-α_1PI antibodies. The gels in *C* contain 5% (vol/vol) anti-PPE antiserum. Anode was to the left for the first dimension of electrophoresis and at the top for the second dimension. (*A*-1) Pooled smokers' BAL fluids; (*A*-2) pooled nonsmokers' BAL fluids; (*A*-3) control (nonsmokers) serum. Note the single α_1PI precipitin peak at the same position in all three gels in *A*, indicating identical electrophoretic mobility of α_1PI in all three fluids. (*B*-1) Pooled smokers' BAL fluids plus PPE; (*B*-2) pooled nonsmokers' BAL fluids plus PPE; (*B*-3) control (nonsmokers) serum plus PPE. Note the presence of free α_1PI migrating toward the anode, followed by complexes of α_1PI and PPE in all three gels in *B*. However, a smaller amount of α_1PI–PPE complex and a greater amount of free α_1PI were present in smokers' BAL fluids incubated with the protease (*B*-1) than was the case with nonsmokers' BAL fluid (*B*-2) or control (nonsmokers) serum (*B*-3). (*C*-1) Pooled smokers' BAL fluid plus PPE; (*C*-2) pooled nonsmokers' BAL fluids plus PPE; (*C*-3) control (nonsmokers) serum plus PPE. (In the *C* gels, the antiserum is directed against PPE.) Note the presence of PPE complexes migrating anodally in all three gels in *C*. However, significantly less PPE complex occurred when smokers' BAL fluid was incubated with the protease (*C*-1), and free PPE could be seen migrating toward the cathode (*C*-1). Larger amounts of complex were present and free PPE was absent in other samples (*C*-2 and *C*-3).

Table 2. Analysis of methionine sulfoxide in α_1PI purified from various sources

Source of α_1PI*	Methionine sulfoxide,† mol/mol inactive α_1PI
Untreated normal serum	0
NCS-treated serum	2.2 ± 0.3
Smokers' BAL fluid	3.8 ± 0.2‡
Nonsmokers' BAL fluid	0

* α_1PI was purified by immunoaffinity chromatography from the pooled samples of BAL fluids obtained from the smokers and nonsmokers listed in Table 1. In addition, human serum that had been oxidized with *N*-chlorosuccinimide (NCS) and untreated serum were also used as sources of pure α_1PI.

† The value obtained for methionine after cyanogen bromide treatment and acid hydrolysis was taken as the amount of methionine sulfoxide originally present in the protein. Mean of at least three experiments (each done in triplicate) ± 1 SD.

‡ Significantly different from nonsmokers' value, $P < 0.05$ (*t* test).

same amount of α_1PI in smokers' BAL fluid was unable to complex all of the added PPE, and free PPE could be detected. Fig. 2 also shows that α_1PI in smokers' BAL fluid did not differ in electrophoretic mobility from α_1PI in nonsmokers' BAL fluid or control serum. This last observation suggests that the inactivated α_1PI in smokers' BAL fluid was not complexed with proteases derived from lung neutrophils, because such complexes differ in electrophoretic mobility from native α_1PI (30).

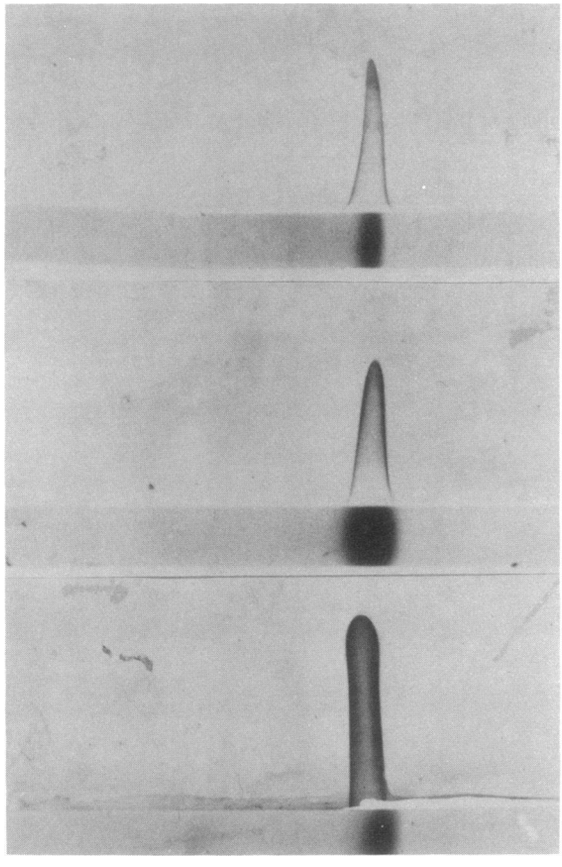

FIG. 3. $NaDodSO_4$ treatment and gel electrophoresis of α_1PI purified from various sources. The samples (75 μg) were boiled in 0.3% $NaDodSO_4$ containing 0.1% 2-mercaptoethanol for 5 min and then subjected to electrophoresis in 10% polyacrylamide gels. Neither the gel nor the electrophoresis buffer contained $NaDodSO_4$. After electrophoresis, the gels were sliced in half and one half was stained with Coomassie brilliant blue G, and the other half (unstained) was subjected to a second electrophoresis (at right angles to the first) into an agarose gel containing 2% (vol/vol) anti-α_1PI antibodies and 1% Triton X-100. Anode is at the left for acrylamide gels and at the top for agarose gels. Gel 1, α_1PI purified from pooled smokers' BAL fluid; gel 2, α_1PI purified from pooled nonsmokers' BAL fluid; gel 3, α_1PI purified from normal nonsmokers' serum.

In addition, immunoreactivity of α_1PI in smokers' BAL fluid was unchanged, because (*i*) smokers' and nonsmokers' α_1PI and control serum α_1PI all showed reactions of identity in double immunodiffusion (Ouchterlony) analysis and (*ii*) equivalent amounts of inhibitor protein from smokers' and nonsmokers' BAL fluids gave identical precipitin rings in radial immunodiffusion analysis (data not shown).

Amino Acid Analysis of α_1PI Purified from BAL Fluid. As shown in Table 2, α_1PI purified from smokers' BAL fluid (see *Materials and Methods*) contained methionine sulfoxide, whereas α_1PI purified from nonsmokers' BAL fluid did not. (Neither sample contained methionine sulfone.) No other statistically significant differences appeared in amino acid compositions of α_1PI purified from smokers' and nonsmokers' BAL fluids compared to α_1PI from control serum (data not shown). Although chemically oxidized α_1PI derived from *N*-chlorosuccinimide-treated serum (7) contained 2 mol of methionine sulfoxide per mol of inactive inhibitor as reported by others (12), 4 mol of methionine sulfoxide were found per mol of inactive α_1PI purified from smokers' BAL fluid (see Table 2).

As shown in Fig. 3, α_1PI purified from BAL fluids of smokers and nonsmokers and treated with $NaDodSO_4$ migrated as a single band upon electrophoresis in polyacrylamide gels. Furthermore, samples of α_1PI derived from smokers' and nonsmokers' BAL fluids and from control serum were indistinguishable by this technique (see Fig. 3). In calibrated $NaDodSO_4$ gels, the calculated molecular weights of the inhibitors from smokers' and nonsmokers' BAL fluids were 52,000 ± 4,500 and 50,000 ± 4,000, respectively ($P > 0.2$, mean of three measurements ± 1 SD). Essentially identical results were obtained when the purified BAL fluid α_1PI fractions were examined by thin-layer gel immunofiltration in Sephadex G-150 (data not shown). Thus, in the foregoing experiments (as well as those shown in Fig. 2), no evidence was obtained to suggest that inactivated α_1PI in smokers' BAL fluid had undergone significant changes in molecular weight as a result of either limited proteolysis or complex formation with proteolytic enzymes.

DISCUSSION

Our findings confirm the observation reported by Gadek *et al.* (10) that α_1PI activity is decreased in cigarette smokers' lung fluids in comparison to similar fluids obtained from nonsmokers. In addition, our studies extend the number of subjects tested and demonstrate that smokers' α_1PI has decreased inhibitory activity against human polymorphonuclear leukocyte elastase as well as PPE. Also, in our study, BAL fluids were obtained repeatedly from several smokers and nonsmoking controls; these samples consistently showed the difference referred to above.

We also show that partially inactivated α_1PI purified from smokers' BAL fluid contains methionine sulfoxide, whereas fully active inhibitor purified from BAL fluid or serum of nonsmokers does not, suggesting that inactivation of lung α_1PI in the smoker is due, at least in part, to oxidation of methionine residues. Although we detected only changes in methionine, our conditions were designed to facilitate methionine sulfoxide quantitation and, conceivably, may have masked additional changes in other amino acid residues. The distribution of methionine sulfoxide between active and inactive α_1PI has not been directly measured; however, the calculated 4 mol of methionine sulfoxide per mol of inactive α_1PI (Table 2) conforms

closely to the value obtained by Wong and Travis (18) for α_1PI purified from rheumatoid synovial fluid. In the latter case, sequence analysis showed that the active-site methionine had been oxidized. Similar studies will be necessary to establish the location of the oxidized methionine residues present in smokers' lung fluid α_1PI.

At least two potential sources of oxidizing activity are present in the smoker's lung and are capable of inactivating α_1PI. One potential pathway of lung α_1PI oxidation in the smoker could be mediated directly by oxidizing free radicals in inhaled smoke (31). In addition to this pathway, oxidants capable of inactivating α_1PI could also be generated in smokers' airways by lung cells (15–17, 32–34). For example, formation of the superoxide radical and H_2O_2 by lung macrophages is increased in smokers (32). Our studies do not permit any conclusions to be drawn regarding which of these mechanisms leads to oxidation of α_1PI in smokers' lungs.

The partially inactive α_1PI present in smokers' BAL fluid could not be distinguished from the active α_1PI present in nonsmokers' BAL fluid on the basis of immunoreactivity, electrophoretic mobility, or molecular weight. Thus, smokers' lung α_1PI appears not to be grossly denatured, inactivated by complex formation with granulocyte proteases (30), or significantly degraded by thiol proteases (35) or by macrophage elastase (36). These latter agents alter the native electrophoretic mobility (30) or molecular weight of the inhibitor (35, 36). Our findings do not support the recent suggestion (based on *in vitro* data) that α_1PI inactivation by cigarette smoke may be due to nonspecific denaturing effects of smoke on lung proteins (37). Our observation that the functional activity of α_1Achy, a protease inhibitor that is resistant to inactivation by oxidation (38), is unchanged in smokers' BAL fluid reinforces this conclusion. Also, at least *in vitro*, previous work (29, 33, 34) suggests that aqueous smoke solutions as well as phagocyte-derived oxidants, at concentrations and incubation times sufficient to partly inactivate α_1PI, do not affect the activity of human neutrophil elastase.

Although the distribution of inactivated α_1PI in the lungs of smokers is not known, it may be likely that the inhibitor is not uniformly inactivated throughout the lung, because cigarette smoke components are not uniformly distributed in the lung (39). In this event, the value of inactive α_1PI measured in smokers' BAL fluid could actually represent the combined mean value from regions in which 100% of lung α_1PI had been oxidatively inactivated and from other areas in which α_1PI had remained fully active. Repeated episodes of such protease–antiprotease imbalance could cause slow deformation of some alveoli and resultant disease in some chronic smokers. Indeed, when the activity of circulating α_1PI is depleted by systemic treatment of experimental animals with a chemical oxidant, connective tissue changes suggestive of the early stages of panacinar emphysema develop (40). However, because increased recruitment of macrophages and neutrophils to the lungs of smokers (1) may also play a role in disrupting protease–anti-protease balance, it will be necessary, in the future, to measure both lung protease burden as well as lung anti-protease function in order to more completely assess this hypothesis.

This work was supported by U.S. Public Health Service Grants HL-14262, 5-T32-07088, CA21930, and HL24653 and Grant 1143 from The Council for Tobacco Research–U.S.A., Inc.

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