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SULFONATE SALTS OF AMINO ACIDS: NOVEL INHIBITORS OF THE SERINE PROTEINASES

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Summary. A series of amino acid-derived sulfonate salts have been synthesized. They were found to inactivate efficiently and selectively human leukocyte elastase. The sulfonate salts of the methyl esters of L-norleucine, L-norvaline and L-valine were the most potent. The enzyme is inactivated irreversibly with concomitant release of bisulfite ion. The results demonstrate for the first time that ionic compounds can indeed function as novel inhibitors for the serine proteinases.

Human alpha-1-proteinase inhibitor (alpha-1-PI) is a defense protein that controls the activity of proteolytic enzymes, particularly that of human leukocyte elastase (HLE) (1-2). The inhibitory activity of alpha-1-PI is drastically reduced when the reactive site methionine residue (Met-358) is oxidized to the sulfoxide form (3-4) by exogenous oxidants such as ozone (5), oxidants in cigarette smoke (6) or, oxygen-derived endogenous oxidants (7-9). The resulting proteinase-proteinase inhibitor imbalance often results in the uncontrolled degradation of lung connective tissue and the development of emphysema (10). This imbalance can be redressed by inhibiting HLE and/or preventing the oxidative inactivation of alpha-1-PI.

This communication reports on the design of a new class of inhibitors of HLE that embody both inhibitory and anti-oxidant activity. The compounds are readily accessible, potent and specific irreversible inhibitors of HLE and are capable of releasing an anti-oxidant under the action of the enzyme.

Materials and Methods

Reagents. Compounds 1 through 9, listed in Table 1, were readily obtained as white, stable and crystalline solids by stirring an aqueous solution of

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Table 1. Inhibition of Human Leukocyte Elastase by Sulfonate Salts, $R\text{-}CH(COOCH_3)NH\text{-}CO\text{-}SO_3^-K^+$

Compound	Precursor Amino Acid	R	k_2/K_i M^{-1} sec^{-1}
1	Gly	H	inactive
2	L-Ala	CH_3	inactive
3	L-Val	$(CH_3)_2CH$	800
4	L-Norval	$CH_3CH_2CH_2$	860
5	D-Norval	$CH_3CH_2CH_2$	inactive
6	L-Norleu	$CH_3CH_2CH_2CH_2$	920
7	L-Leu	$(CH_3)_2CHCH_2$	260
8	L-Met	$CH_3SCH_2CH_2$	260
9	L-Phe	$PhCH_2$	530

potassium metabisulfite with the appropriate amino acid ester isocyanate (11) in dioxane. Purified porcine pancreatic elastase was purchased from Worthington Biochemical Co. BOC-ala-p-nitrophenol and methoxysuccinyl ala-ala-pro-val-p-nitroanilide were obtained from Sigma Chemical Co. Human leukocyte elastase was purchased from Elastin Products Co., St. Louis.

Enzyme Assays and Inhibition Studies. Human leukocyte elastase was assayed as follows: 10 µl of a 3.4×10^{-5} M enzyme solution in 0.05 M sodium acetate buffer, pH 5.5, 20 µl of distilled water or dimethyl sulfoxide and 970 µl of Tris-buffer, pH 7.2, were pipetted into a thermostatted test tube. After equilibration at 25° C, a 100 µl aliquot was transferred to a thermostatted cuvette containing 890 µl Tris-buffer and 10 µl of a 3.15×10^{-2} M solution of methoxysuccinyl-ala-ala-pro-val-p-nitroanilide. The change in absorbance was then monitored at 410 nm for 2 minutes. In a typical inhibition run, 20 µl of a 3.4×10^{-4} M solution of the appropriate inhibitor in either distilled water or dimethyl sulfoxide was mixed with 10 µl of a 3.4×10^{-5} M enzyme solution and 970 µl Tris-buffer placed in a constant temperature bath. 100 µl aliquots were withdrawn at different time intervals and transferred to a cuvette containing substrate (10 µl of a 3.15×10^{-2} M solution), 890 µl of buffer and dimethyl sulfoxide. After incubating for 30 seconds, the absorbance was monitored for 2 minutes at 410 nm. The experiment was repeated using a different inhibitor to enzyme ratio. This ratio usually varied between 10 and 50, depending on the potency of the inhibitor. The method of Kitz and Wilson was then used to analyze the data (12).

Porcine pancreatic elastase and chymotrypsin were assayed as described previously (13-14).

Results and Discussion

Incubation of the monopotassium salt of N-(Sulfocarbonyl)-L-norvaline 1-methyl ester (compound 4 in Table 1), for example, with human leukocyte elastase resulted in progressive loss of enzyme activity. Figure 1 illustrates the time-dependent nature of the inactivation. Similar plots were obtained with the remainder of the active compounds. The k_2/K_i values for the various inhibitors are listed in Table 1. It is apparent from Table 1 that the sulfonate salts of

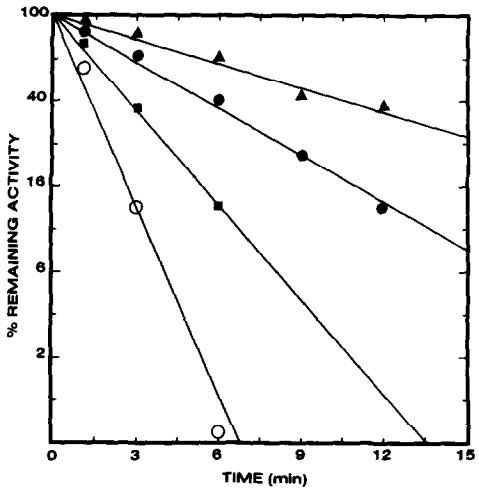

FIGURE 1. Time-dependent inactivation of human leukocyte elastase by compound 4. The enzyme (0.34 μM) was incubated with 4 at 25° C, pH 7.2, with the following concentrations of inhibitor: (▲) 1.7 μM; (●) 3.4 μM; (■) 6.8 μM; (○) 10.2 μM.

L-norvaline, L-norleucine and L-valine methyl ester (compounds 4, 6 and 3, Table 1) are very potent inhibitors of human leukocyte elastase. The inhibitors derived from L-leucine, L-methionine and L-phenylalanine (compounds 7, 8 and 9, Table 1) also inactivate leukocyte elastase but not as efficiently. This is in agreement with what is presently known about the primary specificity site (S_1 subsite) of leukocyte elastase (15). The active site of leukocyte elastase is hydrophobic and prefers a valine, norvaline or norleucine residue at its S_1 subsite (15,16-17). Compound 4 (Table 1) had no effect on chymotrypsin and porcine pancreatic elastase under comparable conditions, attesting to the high specificity of the inhibitor. Thus, selective inhibition can be achieved by manipulating the side chain that binds to the S_1 subsite of each serine proteinase since the topographical features of the active sites of the various serine proteinases are known to be different. As expected the inhibitor derived from D-norvaline methyl ester (compound 5, Table 1) showed no inhibitory activity toward leukocyte elastase. The compounds derived from glycine and L-alanine were also devoid of any inhibitory activity. The lack of inhibitory activity of these compounds can be ascribed to poor binding. We have observed that the initial rapid loss of enzymatic activity is followed by very slow regaining of activity (10% enzyme activity was regained after 7 h). This

suggests that rapid acylation of the active site serine with concomitant release of bisulfite ion, is followed by very slow deacylation of the enzyme.

Human leukocyte elastase prefers hydrophobic substrates and because of that, all inhibitors made in the past took cognizance of this important fact (14). Consequently, the inhibitors reported thus far are highly hydrophobic with limited solubility in aqueous media. The amino acid sulfonate salts demonstrate for the first time that ionic compounds can function as inhibitors of leukocyte elastase, provided the group bearing the charge is on the leaving group side. It is likely that the sulfonate group is not occupying the S_1' subsite but rather is pointing away from the surface of the enzyme and toward the aqueous milieu. The high potency and specificity exhibited by these inhibitors, as well as their ease of preparation, render them highly useful as enzyme probes and pharmacological agents.

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