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INHIBITION OF HUMAN LEUCOCYTE ELASTASE BY FATTY ACYL-BENZISOTHIASOLINONE, 1,1-DIOXIDE CONJUGATES (FATTY ACYL-SACCHARINS)

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Abstract—Derivatives of benzisothiazolinone 1,1-dioxide (saccharin) N-acetylated with aliphatic and aromatic substituted aliphatic acyl groups were prepared. The inhibitory activity of the compounds was assayed against human leucocyte elastase (EC 3.4.21.37) and several other proteases. The IC_{50} values for inhibition of the human leucocyte elastase decreased with increasing length of the acyl residue, and reached a minimum value at C_{16} (2 μM). This phenomenon and the decrease of the inhibition by surfactants or by saturation of the enzyme with palmitic acid, indicates that in addition to acylation, hydrophobic interactions are also involved in the inhibition of this proteinase by compounds substituted with acyl groups containing at least 12 carbon atoms. The inhibitory activity of *N*-palmitoyl-benzisothiazolinone 1,1-dioxide (palmitoyl-saccharin) is about 14 times higher toward human leucocyte elastase than for thrombin (EC 3.4.21.5), and several hundred times, compared to porcine pancreatic elastase (EC 3.4.21.36) and to plasmin (EC 3.4.21.7). Fatty acylated saccharin derivatives were seen to bind in a saturable fashion to insoluble elastin, and decreased the susceptibility of this protein to hydrolysis by human leucocyte elastase.

Human leucocyte elastase (HLE†) is the subject of intense investigation because of its likely involvement in the pathogenesis of pulmonary emphysema and inflammatory disorders [1–3]. Therefore, modulation of its activity offers the promise of effective therapeutic intervention in several disease states. Heterocyclic compounds have been described as powerful serine proteinase inhibitors although lacking specificity against one particular enzyme of this class [4–6]. It was recently demonstrated that many hydrophobic compounds and especially fatty acids could selectively inhibit leucocyte elastase [7–10]. Such interactions probably involve the presence of an unusual hydrophobic binding pocket near the active site of HLE [8, 11]. We therefore took advantage of these findings and coupled to benzisothiazolinone 1,1-dioxides (saccharin), known acylating agents of serine proteinases [4], alkylating compounds (including fatty acids) with pronounced hydrophobic character. We demonstrated that the HLE inhibitory potency of these derivatives increased as a function of their carbon chain length; also, *N*-palmitoyl-benzisothiazolinone 1,1-dioxide (palmitoyl-saccharin) was found to inhibit leucocyte elastase more selectively than butyryl-saccharin and by binding to the substrate itself, it could partially protect elastin against proteolysis catalysed by HLE.

MATERIALS AND METHODS

Human sputum elastase was purchased from the Elastin Products Co. (Pacific, MO, U.S.A.), *Pseudomonas aeruginosa* proteinase was a gift from Prof. Wallach (Lyon, France), all other enzymes and synthetic substrates came from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Saccharin sodium, the organic acids, the acid chlorides and the thionyl choride were from Aldrich Chimie (Strasbourg, France). Dimethoxycinnamoyl chloride was prepared from the acid by the thionyl chloride method [12].

Synthesis of N-*acyl-benzisothiazolinone* 1,1-*dioxides (acyl-saccharins)*. The acylation of the benzisothiazolinone 1,1-dioxide was carried out by a variant of the general procedure described previously [13]. Briefly, 0.012 mol of the acid chloride and 0.01 mol of dry sodium benzisothiazolinone 1,1-dioxide (sodium saccharin) were refluxed for 4 hr in 10 mL of dry tetrahydrofuran with mechanical stirring. The reaction mixture was filtered, the filtrate was concentrated under vacuum, and the product was recrystallized from ethanol and dried in air. Twenty millilitres of tetrahydrofuran were used for the preparation of the dimethoxy cinnamoyl derivative considering the lower solubility of the acid chloride. Yields were between 20 and 40% for the different compounds. No attempt was made to maximize yields.

Melting points, uncorrected, shown in Table 1, were determined on a Koffler block (Prolabo, Paris, France). All compounds were analysed for carbon, hydrogen and nitrogen (Microanalytical Laboratory of the Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, France) and were within 0.5% of calculated theoretical values. ^{1}H NMR spectra obtained in deuterated chloroform on a Varian T60 NMR spectrometer were in full agreement with the expected structure of the synthesized compounds.

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† Abbreviations: Saccharin, benzisothiazolinone 1,1-dioxide; palmitoyl-saccharin, *N*-palmitoyl-benzisothiazolinone 1,1-dioxide; acyl-saccharins, *N*-acyl-benzisothiazolinone 1,1-dioxides; HLE, human leucocyte elastase; -pNA, *p*-nitroanilide; MeOSuc-, *N*-methoxysuccinyl-; PPE, porcine pancreatic elastase.

Table 1. Inhibitory activity of lipophilic saccharin derivatives toward HLE

Compound	N	IC_{50} (μM)	m.p. (°C)
Aliphatic acyl-saccharins			
Acetyl-saccharin	0	270	194
Butyryl-saccharin	2	200	172
Hexanoyl-saccharin	4	33	88
Octanoyl-saccharin	6	74	106
Decanoyl-saccharin	8	24	92
Lauroyl-saccharin	10	8.2	91
Myristoyl-saccharin	12	3.8	94
Palmitoyl- saccharin	14	2.0	99
Stearoyl-saccharin	16	4.5	97
Undecenoyl-saccharin	9	25	80
Aromatic acyl-saccharins			
Series 1: saturated aliphatic side chain			
Phenyl acetyl-saccharin	1	330	184
Phenyl propionyl-saccharin	2	48	154
Phenyl butyryl-saccharin	3	120	124
Phenyl valeryl-saccharin	4	150	140
4-Methoxyphenyl butyryl-saccharin	3	39	150
Series 2: unsaturated aliphatic side chain			
Cinnamoyl-saccharin		31	230
3,4-Dimethoxycinnamoyl-saccharin		12	223
3,4,5-Trimethoxycinnamoyl-saccharin		25	198

Experiments carried out at 45° with Suc-Ala-Ala-Ala-pNA (0.625 mM) as substrate in a pH 8 Tris–HCl buffer containing 0.01% Brij 35.
N = number of methyl groups. m.p. = melting point.

NMR signals for palmitoyl-saccharin: 0.9 (3H, CH_3); 1.10–1.6 (m 26H, 13, CH_2); 2.20 (dd 2H, —CH_2—CO—); 8.00 (m 4H, aromatic). Radiolabelled palmitoyl-saccharin was prepared in a similar manner to the non-radioactive compound, using mmol quantities of the reactants. The [^{3}H]-palmitoyl chloride was prepared as described previously [14] by equilibration of [^{3}H]palmitoic acid (Amersham, TRK 760, 54 mCi/mmol) with an approximately 100-fold excess of palmitoyl chloride. The radiolabelled product was purified by HPLC on a Bondapack C18 column using a gradient (30–100%) of water–acetonitrile containing 0.01% trifluoroacetic acid. The purified compound (5360 cpm/μg) was homogeneous when rechromatographed in the same system.

Assay of the stability of the palmitoyl-saccharin

In the absence of water. One milligram of the palmitoyl-saccharin was dissolved in 5 mL of heptane. Samples were withdrawn after 1, 7 and 30 days. The UV spectra and the HPLC diagrams of the samples were identical with those obtained immediately after dissolution.

In aqueous ethanol. One millilitre of palmitoyl-saccharin was dissolved in 4 mL of ethanol and 1 mL of water was added to the solution. Scanning UV spectroscopy and HPLC were carried out on samples taken immediately after dissolution and 2, 6 and 24 hr later.

Proteinase assays

All proteinases were titrated using α_1 proteinase inhibitor (Sigma No. A9024) which had been standardized with trypsin (EC 3.4.4.21.4) of known activity, determined with *p*-nitrophenyl-*p'*-guanidobenzoate HCl (Sigma) [15]. The peptidolytic activities of HLE (Table 1) and of porcine pancreatic elastase (PPE) (EC 3.4.21.4) were assayed at 45° with Suc-Ala-Ala-Ala-*p*-nitroanilide (pNA) (0.625 mM) in 50 mM Tris–HCl buffer, pH 8 containing 50 mM NaCl and 0.01% Brij 35 [14]. The liberation of the pNA was followed with a PU 8740 Philips recording spectrophotometer. The inhibitors were added in 25 μL acetone to the enzyme solution, the final volume of the reaction mixture was 2 mL. The same quantity of acetone was added to the controls. HLE (17.2 nM) and PPE (34.5 nM) were preincubated with the inhibitors for 10 min at 45° before determination of the residual enzyme activities at the same temperature. To compare the inhibitory activities of the palmitoyl-saccharins toward other serine proteinases, plasmin (60 mU/mL), thrombin (30 mU/mL), trypsin (41 nM), HLE and PPE were assayed at 37° with Tosyl-Gly-Pro-Lys-pNA, Tosyl-Gyl-Pro-Arg-pNA, Bz-Arg-pNA, *N*-methoxysuccinyl-(MeOSuc)-Ala-Ala-Pro-Val-pNA and Suc-Ala-Ala-Ala-pNA, respectively at 0.05 mM substrate concentrations in a 50 mM pH 8.4 Tris–HCl buffer (Table 2). The peptidolytic activity of subtilisin (EC 3.4.21.14) was determined with *N*-*p*-Tosyl-Gly-Pro-Lys-pNA at pH 8, in essentially the same way. The dependence of the initial velocity of the substrate hydrolysis in the presence of increasing inhibitor concentrations was carried out at different substrate concentrations [14–16]. Each value corresponded to

Table 2. Comparison of the inhibitory capacity of the butyryl- and palmitoyl-saccharins toward different serine proteinases, at 37° using 0.05 mM substrate concentrations

	HLE	PPE	Thrombin	Plasmin	*Pseudomonas aeruginosa elastase*
Butyryl-saccharin	190	200	84	NI	NI
Palmitoyl-saccharin	0.07	47	1	80	NI

For experimental details see text.
NI, not inhibitory.

the mean of at least 3 assays, SD <5%. Inhibition is reported (Table 1) as the concentration of inhibitor giving 50% inhibition under the specified conditions.

An attempt was made to determine the rate of inhibition (K_{ass}) by combining HLE with palmitoyl-saccharin and assaying residual proteinase activity at time intervals, as described [15], under pseudo first order (active enzyme to inhibitor ratio of 1:50) and second order (enzyme to inhibitor ratio 1:1), using MeOSuc-Ala-Ala-Pro-Val-pNA as substrate. To assay the recovery of the elastase activity (K_{dissoc}), a solution of the enzyme (20 nM) was incubated with a slight excess (20%) of palmitoyl-saccharin under similar conditions as used for the determination of the inhibitory activities. Proteinase activities were assayed in samples drawn after 12, 24 and 48 hr. The results were corrected for the deactivation of the enzyme under similar experimental conditions. As the half-life of the palmitoyl-saccharin was found to be about 12 hr (see results), the recovery of a fraction of the uninhibited peptidolytic activity can be anticipated as a result of the dissociation of the enzyme–inhibitor complex.

The elastinolytic activities were determined with ^{3}H-labelled ligamentum nuchae bovine elastin (sp. radioact. 17,700 cpm/mg), as detailed elsewhere [14, 16]. Bovine ligamentum nuchae elastin prepared by the Lansing method was a gift from Eurorga (Pantin, France), its purity was controlled by amino acid analysis and by the absence of hexoses and hexosamines.

Adsorption of the palmitoyl-saccharin on elastin was measured by stirring insoluble elastin (32 mg) overnight at 37° in 8 mL of a 0.1 M Tris–HCl pH 8.4 buffer, containing 0.02% NaN_3. Aliquots of this suspension (0.5 mL for the study of the adsorption kinetics and increasing quantities, 0.05–4 mg for the saturation studies) were placed in Eppendorf centrifuge tubes and made up to 1 mL with the Tris buffer. The radiolabelled compounds were added to this mixture dissolved in 10 μL ethanol. The tubes were agitated at 37° for 1, 2, 4, 6 and 24 hr for the kinetic studies and 6 hr for the determination of the effect of the variation of the elastin concentration. Elastin fibers were separated by centrifugation at 12,000 rpm, washed three times with the same buffer, the washings and the supernate were discarded. The elastin was solubilized with 25 μM PPE in 1 mL of the Tris buffer for 24 hr at 37°. The radioactivity of aliquots was determined by scintillation counting. Values were corrected for non-specific interactions with those obtained in the presence of a 200-fold excess of palmitic acid.

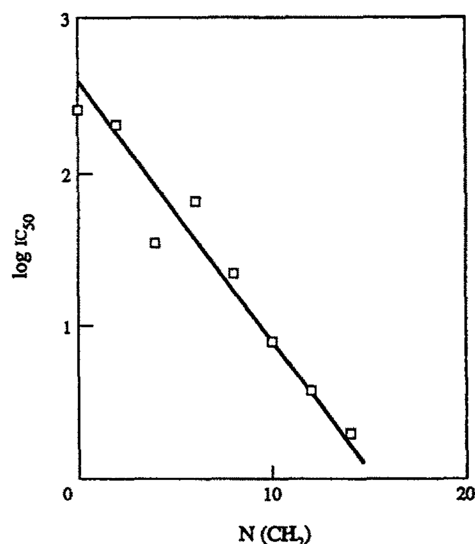


Fig. 1. Number of CH_2 residues in acyl-saccharin, plotted against the logarithm of IC_{50} values. For experimental conditions see Table 1.

RESULTS

The interaction between HLE and acyl-saccharins whatever the acyl substituent (see Table 1), was found to occur within a few seconds at IC_{50} concentration of the inhibitor. Consequently, rates of inactivation (k_{obs}/I) could not be determined without the use of stop-flow equipment and only IC_{50} values are thus reported. Aromatic acyl-saccharins bearing from 0 to 4 CH_2 groups between the saccharin moiety and the phenyl ring were found to be poor inhibitors of HLE with IC_{50} less than 10 μM. The inhibitory capacity of these compounds did not increase with the number of methyl groups but the presence of a methoxy group on the phenyl ring decreased by 4-fold the IC_{50} of phenylbutyryl-saccharin. The inhibitory potential of aliphatic acyl-saccharins was, under our experimental conditions, correlated with their carbon chain length (Table 1, Fig. 1).

Palmitoyl-saccharin, the best HLE inhibitor in this series, was taken as our "pilot" compound and its properties were compared to those of butyryl-saccharin. For both saccharin conjugates, recovery of elastase activity was found to be extremely slow at neutral pH, suggesting that k_{deass} is in the same range as values obtained for other *N*-acyl-saccharins [4, 5]. Also, the inhibition was not reversed rapidly with hydrazine, although 80% recovery of activity at 37° was achieved after complexing HLE with butyryl-saccharin and 60% with palmitoyl-saccharin.

These findings indicate that palmitoyl-saccharin as other *N*-acyl-saccharins [4] inhibited irreversibly HLE by forming a stable acyl-enzyme. The contribution of the fatty acyl moiety to the HLE inhibitory potency of palmitoyl-saccharin was further investigated. In order to show whether occupancy of the HLE hydrophobic domain could influence acylation of the active site of the enzyme, the following experiments were performed. First, palmitic acid was allowed to interact with HLE under conditions where about 50% of the activity of the enzyme was inhibited, then either butyryl-saccharin or palmitoyl-saccharin were added to palmitic acid–HLE complexes. When such experiments were performed with *N*-butyryl-saccharin, the inhibitory effects of both substances were cumulative (Fig. 2). Such an additive effect was not observed when replacing butyryl-saccharin with palmitoyl-saccharin.

The presence of Brij 35 in the assay buffer was found to have a major effect on the HLE inhibitory potency of palmitoyl-saccharin e.g. a nearly 20-fold increase of the IC_{50} value. It did not, however, affect appreciably HLE–butyryl-saccharin interactions (see Tables 1 and 2). Also, decreasing the temperature from 45 to 37° diminished by 80% the potency of palmitoyl-saccharin. Altogether, these data indicate a dual role for the aliphatic chain (via hydrophobic interactions with S_3–S_5 subsites of HLE) and the heterocycle (via acylation of the serine active site) in the HLE inhibitory mechanism of these *N*-fatty acyl-saccharins.

The inhibitory capacities of *N*-butyryl- and *N*-palmitoyl-saccharin towards several proteinases were studied under similar experimental conditions: temperature, substrate and enzyme concentrations (Table 3); the palmitoyl-saccharin conjugate was found to be a much better inhibitor for the serine proteinases investigated than its butyryl counterpart. Importantly, at least under our experimental conditions, it inhibited HLE with IC_{50} values of 14-, 658- and 1020-fold lower than thrombin, PPE and plasmin, respectively. In contrast, butyryl-saccharin inhibited HLE, PPE and thrombin with similar IC_{50} values in the 0.8–2 × 10^{-4} M range. *Pseudomonas aeruginosa* elastase activity was not affected by these substances.

It was recently shown that the association rate constants for several HLE irreversible inhibitors are decreased 30- and 60-fold in the presence of fibrous elastin [17]. We therefore analysed the influence of this fibrous substrate on the inhibitory capacities of lauroyl- and palmitoyl-saccharins. The inhibitors were either incubated with HLE before adding elastin or allowed to interact with elastin fibres prior to adding the enzyme. The kinetics of elastolysis are represented in Fig. 3a and show that elastin degradation was much less pronounced under conditions where the substrate can directly compete with the acylating agent for HLE binding. Dose–effect relationships were studied under the two sets of experimental conditions as shown in Fig. 3b. When inhibitors and enzyme were preincubated prior to addition of elastin, IC_{50} values for palmitoyl- and lauroyl-saccharins were equal to 0.7 μg/mL (1.7 μM) and 24 μg/mL (66 μM), respectively. These figures have to be compared with inhibitory capacities obtained with an oligopeptide substrate, 2 and 8.2 μM respectively (see Table 1), although under slightly different experimental conditions. If the inhibitor and elastin were mixed before supplementing the solution with enzyme the inhibitory capacity of both saccharin conjugates was lowered.

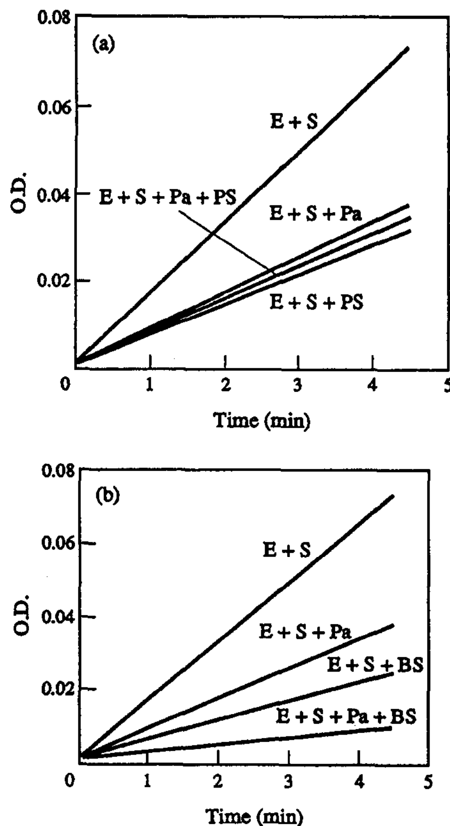
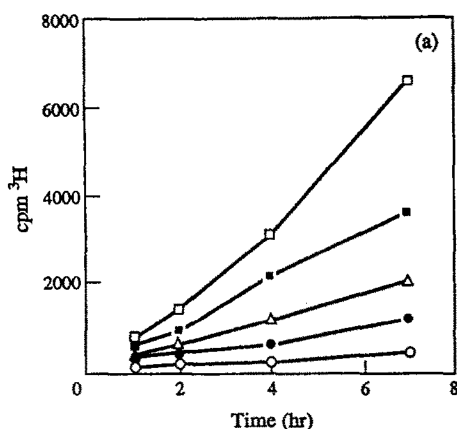


Fig. 2. Effect of the occupation of the hydrophobic site of the HLE by palmitic acid on the inhibition of the hydrolysis of Suc-Ala_3-pNA (S) by palmitoyl- and butyryl-saccharins. Time course of the substrate hydrolysis. (a) E + S = substrate (S) digested by HLE (E); E + S + Pa = digestion of the substrate in the presence of palmitic acid; E + S + Pa + PS = digestion of the substrate in the presence of palmitic acid and of palmitoyl-saccharin; E + S + PS = digestion in the presence of palmitoyl-saccharin. (b) E + S + BS = digestion in the presence of butyryl-saccharin; E + S + Pa + BS = digestion in the simultaneous presence of palmitic acid and of butyryl-saccharin.



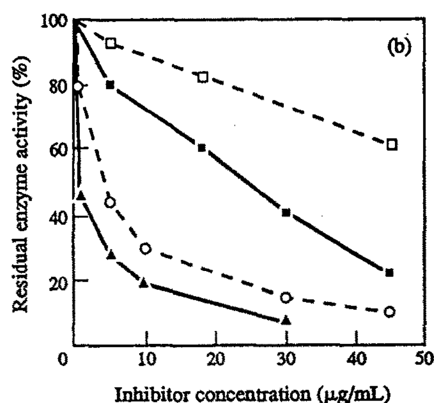
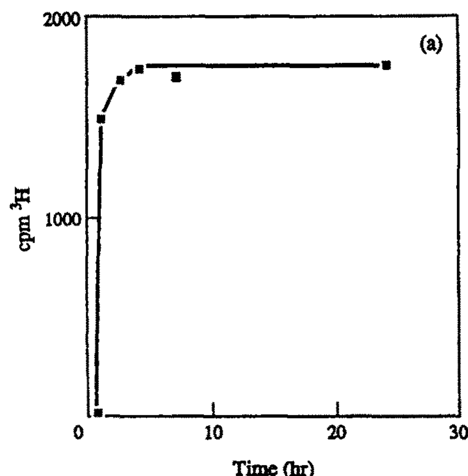


Fig. 3. Kinetics of the hydrolysis of [^{3}H]elastin in the presence of lauroyl- and palmitoyl-saccharin. (a) [^{3}H]-Elastin (75 µg/mL) was treated with 1 µg/mL of HLE in 1 mL of 0.1 M Tris–HCl at pH 8.4 in the presence of 10 µg/mL of inhibitor. The non-digested elastin was separated by centrifugation and the radioactivity of the supernate plotted against the reaction time. Elastin digested without inhibitor (□); elastin preincubated for 30 min with the inhibitor before addition of HLE: palmitoyl-saccharin (■), lauroyl-saccharin (△). HLE preincubated with the inhibitor for 10 min before the addition of elastin, digestion in the presence of palmitoyl-saccharin (○), lauroyl-saccharin (●). (b) Digestion of fibrous elastin with HLE, residual enzyme activities in the presence of increasing inhibitor concentrations. Dotted lines: inhibitor incubated for 30 min with elastin before the addition of the HLE, lauroyl-saccharin (□), palmitoyl-saccharin (○). Continuous lines: HLE incubated for 10 min with the inhibitor before addition of the elastin, lauroyl-saccharin (■), palmitoyl-saccharin (△).



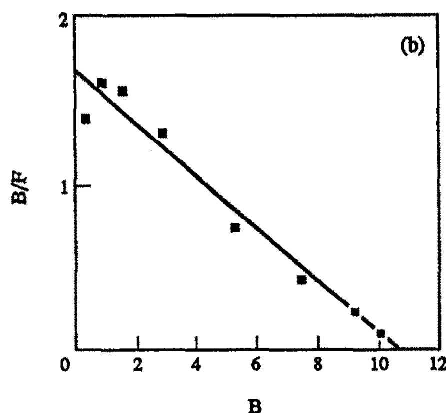


Fig. 4. Adsorption of palmitoyl-saccharin on fibrous elastin. (a) Time dependence. Elastin (2 mg) was incubated with 10 µg of [^{3}H]palmitoyl-saccharin in 1 mL of Tris–HCl pH 8.4 buffer and the sample was continuously shaken. The elastin was separated by centrifugation after 0.5, 2, 4, 6 and 24 hr, washed with the same buffer and the radioactivity of the elastin was determined after solubilization with PPE. (b) Scatchard plot for the fixation of the [^{3}H]palmitoyl-saccharin on elastin. Increasing quantities of elastin (0.1–4 mg) were incubated with 10 µg of [^{3}H]palmitoyl-saccharin under experimental conditions indicated in (a). Values obtained in the presence of a large excess 500 µg of palmitic acid were deduced from those obtained with palmitoyl-saccharin alone. F = free palmitoyl-saccharin, B = bound palmitoyl-saccharin (nM/µg elastin).

The inhibitory capacity (IC_{50}) of the palmitoyl-saccharin was increased 4-fold and 50% inhibition of elastolysis was not or hardly attained even with the highest concentration of lauroyl-saccharin. We therefore confirm previous results of Padrines and Bieth [17] showing that fibrous elastin could influence the potency of irreversible inhibitors, probably by influencing the values of k_{ass}. In this respect fatty acyl-saccharins may present advantages as compared to other irreversible HLE inhibitors by binding to elastin and protecting against elastolysis.

We analysed quantitatively the interactions between [^{3}H]palmitoyl-saccharin and insoluble ligamentum nuchae elastin. The kinetics of adsorption of the lipophilic saccharin conjugate on elastin is represented on Fig. 4a. It shows that maximal binding of palmitoyl-saccharin to elastin was found saturable with a maximum amount of 10.7 nmol of compounds adsorbed to 1 mg of fibrous elastin. When binding data were analysed according to Scatchard, the presence of only one type of low affinity binding sites ($K = 6.7 \times 10^{-5}$ M) was apparent (Fig. 4b).

Table 3. Comparison of the inhibitory capacity and of the elastoprotecting effect of fatty acid-substituted bifunctional elastase inhibitors

Compound	K_d(M)	A_{max}	Protective effect against elastolysis
Palmitoyl-saccharin	6.5×10^{-5}	10.2	56*
Oleoyl-heparin	4.6×10^{-6}	0.5	88.4† [18]
Oleoyl-Ala_2-Pro-Val	ND	10	73*; 73† [14]

* HLE.
† Murine leucocyte elastase.
A_{max}, maximum adsorption on fibrous elastin (nmol/mg).
ND, not determined.

Accordingly, insoluble elastin was saturated with palmitoyl-saccharin, excess of unbound fatty acyl-saccharin conjugate was removed, and rates of elastolysis of treated and untreated elastin by 1 μg of HLE were compared (Table 3). It was found that such pretreatment conferred 56% protection to the substrate against its degradation by HLE. Such a protective effect is slightly less than that obtained with other bifunctional elastase inhibitors such as a lipopeptide or a lipophilic glycosaminoglycan derivative [18].

DISCUSSION

Previous studies have demonstrated that the amide bond in the heterocyclic ring of acyl-saccharin is cleaved by elastases producing long-lived acyl-enzymes [4, 5]. These substances were found to inhibit equally well HLE and PPE as well as other serine proteinases. Although aryl-saccharins were not quite as active as their acyl counterparts, they were found to inhibit HLE and bovine chymotrypsin without affecting, to a great extent, the activity of several closely related proteinases [4, 5]. Recent data in the literature also indicated that the extended substrate binding domain of HLE can accommodate a large variety of hydrophobic lipids, not only fatty acids [8, 9], 2 chloro-4-(1-hydroxy octadecyl) benzoic acid [19], 6-alkyl-3-(ω-carboxyalkyl)-2-pyrones [20] but also polycyclic molecules such as non-steroidal anti-inflammatory drugs [20] and hydrophobic polyanionic chelators [21]. It was therefore reasonable to assume that increasing the hydrophobic character of the aliphatic chain of *N*-acyl-saccharins would increase their inhibitory potency toward HLE as well as their specificity for this enzyme. Indeed, it was found that the inhibitory capacity of *N*-acyl-saccharins increased exponentially with the carbon chain length of the *N*-acyl substituents, maximum inhibitory potential being found, in the series we synthetized, by *N*-palmitoyl-saccharin.

In contrast with previous studies where only unsaturated fatty acids were effective inhibitors of HLE [9], we found that the corresponding saturated fatty acid series could also inhibit HLE but to a lesser extent. Probably several factors can influence the occupancy of the HLE hydrophobic pocket such as temperature, non ionic detergents [8] and also Cu^{2+} and Zn^{2+} [20] which can modify the interaction between the carboxylate end group of fatty acids and Arg^{217} in the S_5 subsite of HLE [20]. The active site serine of HLE could still be acylated by butyryl-saccharin, but not by palmitoyl-saccharin, when the hydrophobic binding site of HLE was occupied by palmitic acid. This finding further supports previous data [8, 11, 20], suggesting the proximity of this hydrophobic binding site to the active site of HLE. According to the above-mentioned experiment the hydrophobic pocket should be located at a distance greater than 5 Å (size of a butyryl chain) and as the fatty acid could thus insert in a more or less tight fit depending on the type of fatty acids (and its conjugation) to S_2–S_5 subsites of elastase.

Although palmitoyl-saccharin could inhibit PPE, thrombin and plasmin, the IC_{50} (HLE)/IC_{50} (serine proteinases) ratios were 1–2 orders of magnitude lower than those determined with the butyryl counter-part. These data are in agreement with our original proposal suggesting that the incorporation of alkyl substituents which can accommodate the unusual hydrophobic binding of this enzyme would increase the specificity of saccharins as elastase inhibitors. Such an approach could be advantageously exploited with other heterocyclic acylating agents [7, 8].

The advantage of lipophilic elastase inhibitors as compared to other HLE effectors relies on the ability of these compounds to bind to elastin and therefore to offer a possible *in vivo* prophylactic protection of elastic fibres against degradation by leucocyte elastase. This principle has been demonstrated for at least one lipopeptide, oleoyl Ala-Ala-Pro-Val which could inhibit the development of emphysematous and haemorrhagic lesions induced in mice by intratracheal instillation of leucocyte elastase [22].

Although the Ala-Ala-Pro-Val peptide sequence was found to be resistant to hydrolysis by *Pseudomonas aeruginosa* elastase [23], it could be degraded by other proteolytic enzymes (unpublished observations). We therefore turned our attention to other lipophilic conjugates and recently described the properties of fatty acyl glycosaminoglycans as HLE inhibitors. Whatever the lipophilic conjugate and its mode of binding to HLE, it was shown that fibrous elastin could decrease its inhibitory potency, in agreement with previous findings of Padrines and Bieth [17]. However, it has to be emphasized that

palmitoyl-saccharin could still be considered as a "good" elastase inhibitor when elastin is used as substrate with IC_{50} equal to 1.7 μM. Also, saturation of elastin with this compound conferred upon this macromolecular substrate a partial resistance to further elastolysis by HLE. In aqueous solutions, palmitoyl-saccharin was much more stable (half life = 12 hr) than furanoyl-saccharin (a few minutes [4, 5]), which nevertheless was found to be efficient in preventing emphysema in animal models [2].

Our results further support the potential use of fatty acyl-saccharins as inhibitors of HLE in those pathological conditions where this enzyme plays an important role.

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