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Inhibition of Lipoprotein Lipase by Alkanesulfonyl Fluorides

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Abstract—A number of alkanesulfonyl halides (chlorides and fluorides) and esters were synthesized and their effect on the activity of lipoprotein lipase (LPL) was studied. Sulfonyl fluorides proved to be efficient inhibitors of LPL when the enzyme was incubated with a 10-fold molar excess of the inhibitors in a buffer containing bile salts (deoxycholate). Hexadecane- and dodecanesulfonyl fluorides caused 50% inhibition of LPL activity at concentrations of 10 to 20 μM.

Plasma lipoproteins are spherical molecular aggregates composed of a core of insoluble lipids (triacylglycerols and cholesteryl esters) surrounded by a surface layer of more polar lipids (phospholipids and cholesterol) and specific lipid-binding proteins (apolipoproteins).[1] Chylomicrons are made from dietary lipids in the intestinal mucosal cells, while the smaller very low density lipoproteins are made in the liver from endogenous lipids. The major step in catabolism of these lipoproteins is the hydrolysis of their triacylglycerols by lipoprotein lipase (LPL).[2] In addition, LPL has been found to efficiently mediate binding of lipoproteins to cell surfaces, to extracellular matrix and to the low density lipoprotein receptor-related protein under cell culture conditions.[3,4] This bridging effect is not dependent on the catalytic activity of LPL, but follows from the ability of LPL to simultaneously interact with proteoglycans/receptors and with lipoproteins. Experimental studies of the noncatalytic functions of LPL require inactivated enzyme.[5] This may be produced by blocking its active site with specific inhibitors like tetrahydrolipstatin or hexadecanesulfonyl fluoride.[6–8] Tetrahydrolipstatin (Orlistat®) was originally developed as a drug to inhibit digestive lipases (pancreatic and gastric lipases) for the purpose of reducing fat absorption. It is now a registered drug for weight reduction. Tetrahydrolipstatin causes almost complete inhibition of LPL, but it presents the drawback of slow reversibility since the drug is slowly turned over by the enzyme.[5,6] By having high concentrations of the inhibitor present during the experiments, any LPL that regains activity will be immediately inhibited by a new inhibitor molecule.[8] Hexadecanesulfonyl fluoride has the advantage of causing irreversible inhibition of LPL.[5,7] It has, however, the disadvantage of decreasing the solubility of the enzyme, probably because two long aliphatic chains from the inhibitor are added per LPL dimer.[5] The short-chain variant, phenylmethanesulfonyl fluoride (PMSF) was previously reported to inhibit LPL,[5,9] but the concentrations required of the inhibitor are in the mM range and in our hands the inhibition is incomplete. To develop more efficient inhibitors, suitable for the study of the noncatalytic functions of LPL, we have synthesized a number of alkanesulfonyl halides, of short, medium and long aliphatic chains, and esters, and studied the inhibition of LPL activity caused by these compounds.

Chemistry

Hexane-, dodecane- and hexadecanesulfonyl chlorides and fluorides were used in this study. Hexane- and dodecanesulfonyl chlorides (**3**, **4**) were prepared in good yield by the treatment of commercially available sodium sulfonates (**1**, **2**) with *N*-chlorosuccinimide in the presence of triphenylphosphine under mild conditions (room temperature, 1 h), whereas hexadecanesulfonyl chloride (**5**) is commercially available. This mild chlorination method is applicable even to complex substrates containing other sensitive functional groups. Alkanesulfonyl fluorides **6**–**8** were obtained from the

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corresponding chlorides **3**–**5** by refluxing in anhydrous acetone for 3 h, in the presence of a 10-fold molar excess of anhydrous sodium fluoride.[10]

Two esters of hexanesulfonic acid have been chosen to be prepared and tested for their ability to react with the active site of LPL. *p*-Nitrophenyl hexanesulfonate (**9**) is an analogue of *p*-nitrophenyl phosphonates, which have been proven potent inhibitors of digestive lipases[11] and microbial lipases.[12,13] *N*-[(Hexanesulfonyl)oxy]succinimide (**10**) was chosen because derivatives of *N*-(sulfonyloxy)succinimide have been reported to inhibit the serine proteases human leukocyte elastase[14] and chymotrypsin.[15] LPL is also a serine hydrolase with a catalytic triad consisting of Ser-132, Asp-156 and His-241.[5] Compound **9** was prepared by addition of hexanesulfonyl chloride (**3**) to a solution of *p*-nitrophenol and NaH in THF at −30 °C, followed by stirring for three days at room temperature. For the synthesis of compound **10** a solution containing equimolar amounts of *N*-hydroxysuccinimide and compound **3**, and a few drops of pyridine in CH_2Cl_2 was refluxed for 1 h.

All products gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.[16]

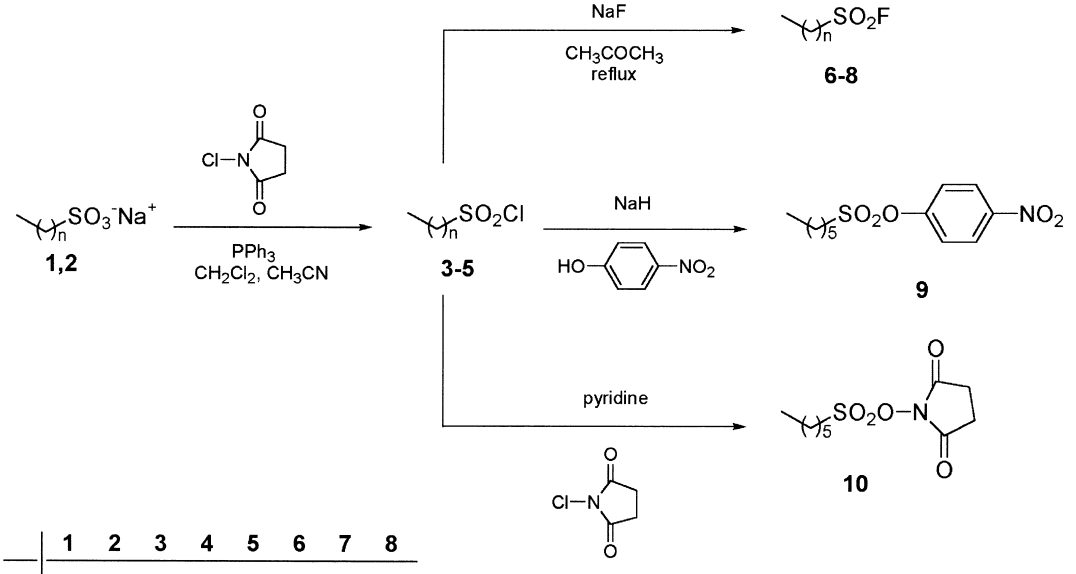
Inhibition Studies

LPL purified from bovine milk[17] (500 μg/mL in 5 mM deoxycholate, 10 mM Tris–HCl, pH 8.5)[5] was preincubated at room temperature with the inhibitor at a ratio calculated on the concentration of LPL monomer (50 kDa). LPL alone was virtually stable under the conditions used. The inhibitors were dissolved and diluted in DMSO. The final concentration of DMSO in the mixtures with LPL was 1% (v/v). Control experiments showed that this concentration per se did not affect the activity of LPL. Samples (5 μL) were taken at the indicated times for assay of remaining LPL activity. As an adaptation to the linear range of the assay, each sample had to be immediately diluted 400-fold (in 2 mL cold 5 mM deoxycholate, 0.1% sodium dodecyl sulfate, 10 mM Tris–HCl, pH 8.5) before assay, and 5 μL of the diluted material was then used for the incubation with the lipid substrate. The assay system contained in a total volume of 0.2 mL (pH 8.5): 5 mg triglycerides/mL (from radiolabelled Intralipid), 0.1 M NaCl, 0.15 M Tris–HCl, 0.1 mg heparin/mL, 60 mg bovine serum albumin/mL and 5% (v/v) rat serum.[18] The incubations were carried out in duplicate for 15 min at 25 °C.

Porcine pancreatic lipase (PPL, Sigma, type VI-S) in 1 mM Tris–HCl, pH 8.0, containing 0.1 M NaCl and 5 mM $CaCl_2$, was preincubated with the inhibitor (dissolved in DMSO) at a ratio calculated on active enzyme. The preincubation solution contained 3% DMSO. Residual PPL activity was measured with the pH-stat method at pH 8, in a mixture containing: 10.5 mL 1 mM Tris–HCl with 0.1 M NaCl and 5 mM $CaCl_2$, 4 mL 15 mM deoxycholate and 0.5 mL tributyrin.[19] The preincubations and measurements of catalytic activity were carried out at 37 °C. The results are mean values from three identical experiments.

Results and Discussion

Figure 1 shows the time courses for inhibition of LPL by alkanesulfonyl fluorides **6**–**8** and esters **9** and **10** at a 10-fold molar excess of the inhibitors. All sulfonyl fluorides proved to be efficient inhibitors of LPL. After 24 h the remaining enzymatic activity was zero (compounds **7**, **8**) or almost zero (compound **6**). The potency of the inhibitors decreased as the chain length decreased. At 3 h compound **8** (hexadecanesulfonyl fluoride) had inhibited more than 90% of the LPL activity, while compound **6** (hexanesulfonyl flouride) had inhibited only 60% of the activity. On the contrary, the sulfonyl esters (**9** and **10**) were almost inefficient, causing significant inhibition under the conditions used.



	1	2	3	4	5	6	7	8
n	5	11	5	11	15	5	11	15

The inhibitory effect of alkanesulfonyl chlorides was compared to that of the corresponding fluorides, as shown in Figure 2. A 25-fold molar excess of the inhibitors **3**, **4**, **6**, and **7** was used. It is clear that sulfonylchlorides were quite inefficient as inhibitors in comparison with the fluorides. After 90 min of incubation, dodecanesulfonyl fluoride completely inhibited LPL, while the corresponding chloride caused less than 10% inhibition.

The dose-dependent inhibition of LPL catalytic activity by alkanesulfonyl fluorides was studied. Plots of remaining LPL activity versus concentration of inhibitors **6**–**8** are shown in Figure 3. In this experiment the concentration of LPL monomer was about 5-fold lower than in the previous experiments (2 μM as compared to 10 μM).

Hexadecane- and the dodecanesulfonyl fluorides caused 50% inhibition of LPL at concentrations of 10 to 20 μM. The decrease of the chain length from 16 to 12 carbon atoms caused a slight decrease in the inhibitory power. A decreased chain length to six carbon atoms (compound **6**) resulted, however, in that about a 10-fold higher concentration of the inhibitor was required for 50% inhibition of LPL.

LPL and PL have about 30% sequence identity, suggesting a similar tertiary fold.[20] Despite the considerable sequence homology, they display some important functional differences, for example substrate specificity[2] and affinity to heparin-like molecules and lipoproteins.[21] Three-dimensional models have been proposed to explain the structural determinants of LPL, which are responsible for heparin binding, dimer formation and phospholipase activity.[20] The alkanesulfonyl halides **3**–**8** and esters **9** and **10**, studied with LPL, were also studied for their ability to inhibit PPL using the pH-stat method. Among the compounds tested in this study, only dodecanesulfonyl and hexadecanesulfonyl chlorides **4** and **5** inhibited PPL activity at a high enzyme:inhibitor molar ratio (1:250). Fig. 4 shows the dependency of the remaining PPL activity as a function of time for compounds **4** and **5**. These results are in agreement with the findings that short chain alkanesulfonyl fluorides did not inhibit PPL, while aromatic sulfonyl chlorides may inhibit PPL if they contain electronegative substituents in the ring.[22,23]

Taking into consideration that the common serine hydrolase inhibitor phenylmethanesulfonyl fluoride inhibits LPL only at high concentrations (mM) and the inhibition is usually not complete,[5] it seems that for efficient inhibition of LPL linear alkanesulfonyl fluorides with at least medium chain lengths (12 carbon atoms) are needed. Hexanesulfonyl fluoride is also possible to use, but a higher ratio of inhibitor to LPL is needed for efficient inhibition. In all experiments with LPL a detergent-containing buffer (5 mM deoxycholate) was necessary for efficient inhibition. It is not known whether the detergent is required for solubilization of the inhibitors, or whether it affects the conformation of LPL so that its active site is accessible to the inhibitors. A similar experience was previously made with tetrahydrolipstatin as an inhibitor for LPL.[5,6]

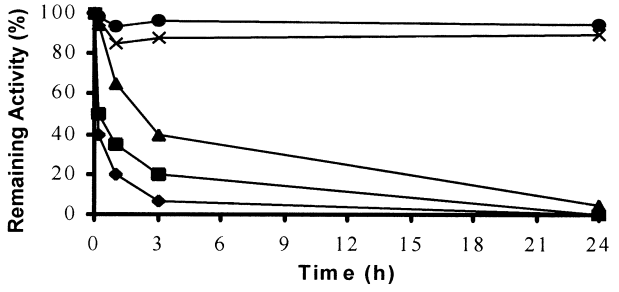


Figure 1. Time courses for inhibition of LPL by alkanesulfonyl fluorides and esters (-◆- **8**, -■- **7**, -▲- **6**, -x- **10**, -●- **9**). LPL and inhibitors were incubated at a molar ratio 1:10 (calculated on LPL monomer) in 10 mM Tris–HCl, 5 mM deoxycholate, pH 8.5 at room temperature.

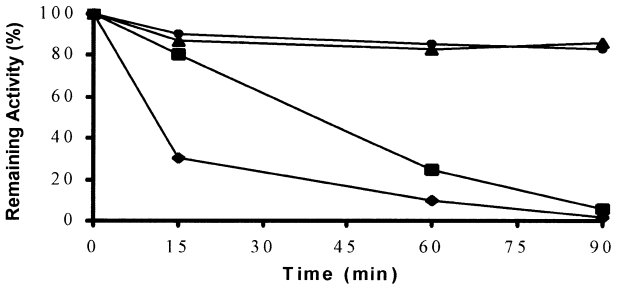


Figure 2. Time courses for inhibition of LPL activity by alkanesulfonyl fluorides and chlorides (-◆- **7**, -■- **6**, -▲- **4**, -●- **3**). LPL and inhibitors were incubated at a molar ratio 1:25 in 10 mM Tris–HCl, 5 mM deoxycholate, pH 8.5 at room temperature.

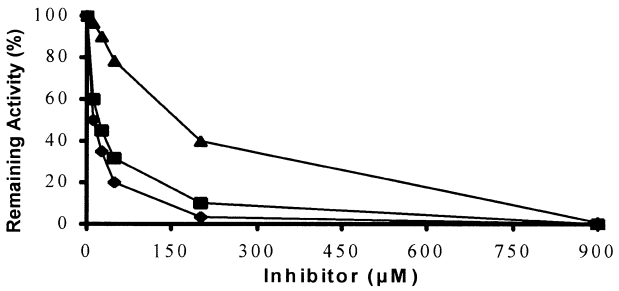


Figure 3. Dose-dependent inhibition of LPL catalytic activity (monomer concentration 2 μM) by increasing concentrations of alkanesulfonyl fluorides (-◆- **8**, -■- **7**, -▲- **6**). In this experiment the incubation time with the inhibitors was 60 min at room temperature.

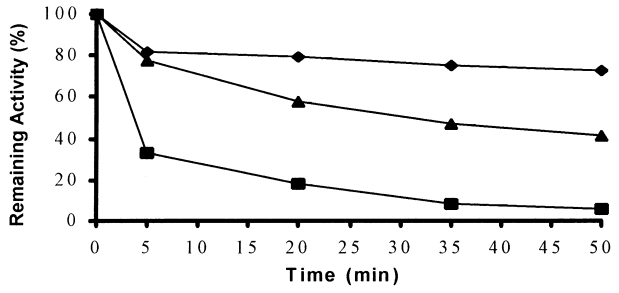


Figure 4. Time courses for inhibition of PPL by alkanesulfonyl chlorides. PPL was preincubated with inhibitor **4** at molar ratios 1:250 (-■-), 1:100 (-◆-) and with inhibitor **5** at a molar ratio 1:250 (-▲-), pH 8 at 37 °C.

Acknowledgements

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16. For example: Compound **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.4 (m, 2H, CH_2SO_2F), 1.95 (m, 2H, $CH_2CH_2SO_2F$), 1.5 (m, 2H, $CH_2CH_2CH_2SO_2F$), 1.3 (m, 16H, $8\times CH_2$), 0.95 (t, 3H, CH_3, $J=6.8$ Hz). Analysis for $C_{12}H_{25}SO_2F$ (252.39): calcd C 57.11, H 9.98; found C 57.21, H 10.09. Compound **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.35 (d, 2H, $J=10$ Hz, 2-H, 6-H), 7.45 (d, 2H, $J=10$ Hz, 3-H, 5-H), 3.4 (m, 2H, CH_2SO_2), 2.05 (m, 2H, $CH_2CH_2SO_2$), 1.5 (m, 2H, $CH_2CH_2CH_2SO_2$), 1.4 (m, 4H, $CH_3CH_2CH_2$), 0.9 (t, 3H, CH_3, $J=9$ Hz). FAB MS: *m/z* 310 (M + Na, 5%), 288 (M + H, 100), 272 (20), 257 (30), 242 (5), 166 (16). Compound **10**: mp 70–72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.55 (m, 2H, CH_2SO_2), 2.9 (s, 4H, $OCCH_2CH_2CO$), 2.05 (m, 2H, $CH_2CH_2SO_2$), 1.55 (m, 2H, $CH_2CH_2CH_2SO_2$), 1.35 (m, 4H, $CH_3CH_2CH_2$), 0.9 (t, 3H, CH_3, $J=6.3$ Hz). FAB MS: *m/z* 286 (M + Na, 3%), 264 (M + H, 40), 165 (2), 116 (100). Analysis for $C_{10}H_{17}NO_5S$ (263.32): calcd C 45.61, H 9.22, N 3.46%; found C 45.71, H 9.00, N 3.47%.
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