Rational Modification of Human Synovial Fluid Phospholipase A_2 Inhibitors†

M. Teresa Pisabarro,*,‡,§ Angel R. Ortiz,‡,§
Albert Palomer,*,∥ Francesc Cabré,∥ Luisa García,∥
Rebecca C. Wade,‡ Federico Gago,§
David Mauleón,∥ and Germano Carganico∥

European Molecular Biology Laboratory, Meyerhofstrasse 1, 69012 Heidelberg, Germany, Departamento de Fisiología y Farmacología, Universidad de Alcalá de Henares, 28871 Madrid, Spain, and R & D Department, Laboratorios Menarini S. A., Alfonso XII 587, 08912 Badalona, Spain

Received September 9, 1993

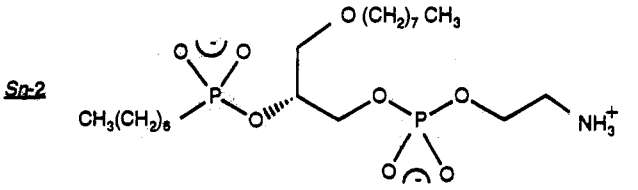
Mammalian nonpancreatic secretory phospholipase A_2 (PLA_2) splits the 2-acyl bond in 1,2-diacylphosphatides.[1] This enzyme has been found in high concentrations in the synovial fluid of patients with rheumatoid arthritis,[2] and it has been suggested that inhibitors of this enzyme may have therapeutic value. The three-dimensional structure of human synovial fluid PLA_2 (HSF-PLA_2) is known both in its native form[3] and in a complex with the transition-state analogue (TSA) L-1-*O*-octyl-2-heptylphosphonyl-*sn*-glycero-3-phosphoethanolamine,[4] **1**. The present work is a part of our program to develop PLA_2 inhibitors and describes the successful rational modifications introduced into **1** aimed at enhancing its affinity toward HSF-PLA_2, based on the combined use of biochemical information, molecular graphics analysis,[5] molecular orbital[6] and molecular mechanics calculations,[7] and the GRID[8] and LUDI[9] programs.

Hydrocarbon chain length is a critical factor for the activity of potential PLA_2 inhibitors. Studies with phospholipid analogues demonstrated that 10 carbons are required in the *sn*-2 acyl chain for optimum binding to cobra venom PLA_2,[10] whereas the optimal length for the *sn*-1 alkyl chain is four carbons in the case of porcine pancreatic PLA_2.[11] These findings can be rationalized in terms of the observed number of contacts between the phospholipid analogs and the enzyme in known PLA_2–inhibitor complexes.[12] Analysis of the HSF-PLA_2 structure with the GRID program suggests similar structure–activity relationships[13] (Figure 1).

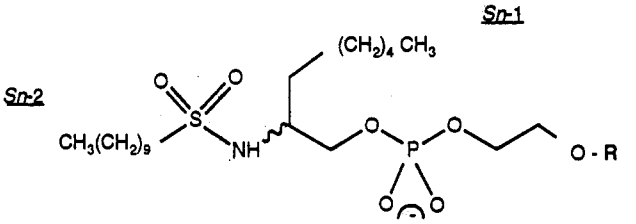
The capacity of TSAs to bind with high affinity has been shown by Gelb *et al.* who introduced a phosphonate group into compound **1**.[14] In contrast, the substitution of acyl by sulfonyl, which is extensively used as a TSA of an ester group undergoing hydrolysis, has been reported by de Haas *et al.*[15] not to improve inhibitory properties. Despite this discouraging data, we decided to introduce the sulfonamide group on the basis of the following rationale: Yu and Dennis[16a] showed that the pK_a of the catalytically active His-48 is 6.1. Therefore, this residue is predominantly unprotonated under physiological conditions. Thus, in order for a TSA to function effectively at physiological pH, the bioisostere of the ester should be chosen so that, in addition to possessing tetrahedral features to resemble the transition state, it has a proton available to form a hydrogen bond to the Nδ atom of His-48, as this hydrogen bond has been shown to provide 1.5 kcal/mol of binding energy.[16a] Monosubstituted sulfonamides have a range of pK_a values that fulfills this requirement at physiological pH.[16b] Moreover, the sulfonamide group may release some strain energy in the molecule. The C–O–P–C dihedral angle of the methylphosphonate moiety of **1** in the complex is 121.2°, giving rise to a strain energy of 0.6 to 0.9 kcal/mol[17] (Figure 2). In contrast, the C–N–S–C dihedral angle of the *N*-methylmethanesulfonamide group has a global energy minimum at 120.0° [18] (Figure 2). These data indicate that sulfonamide-based inhibitors could be at least as effective as the phosphonate-based ones. Accordingly, 2-((decylsulfonyl)amino)-1-octylphosphoglycol **2**,[19] which fulfills the chain length features described above and has a sulfonamide group, is an effective inhibitor of HSF-PLA_2 activity with an $X_i(50) = 0.026$[20] in a mixed vesicle model. In this model, compound **1** inhibited the enzyme with an $X_i(50)$ value of 0.025. Therefore, sulfonamide-based TSAs are effective PLA_2 inhibitors. A molecular model accounting for the interaction of compound **2** with HSF-PLA_2 was built.[21] In this model, carbons 8–10 of the *sn*-2 acyl chain fit in a hydrophobic pocket within the hydrophobic channel[22] surrounded by residues Ala-18, Ala-19, Leu-2, Val-3, Phe-5, and His-6. There are no large conformational differences between this complex and the X-ray structure of HSF-PLA_2 + **1** (rms (Cα) = 0.53 Å; rms (all non-hydrogen atoms) = 3.1 Å).

The result obtained with **2** encouraged us to design new modifications. Thus, the modeled complex of HSF-PLA_2 with **2** was used to search with the GRID program[23] for additional ligand binding sites in the enzyme that could be exploited by further modification of **2**. Favorable aromatic interactions were found within what we have termed the "hydrophobic cage", a hydrophobic pocket delimited by residues Val-46, Thr-130, Pro-131, Gly-33 and the disulfide bridge linking Cys-50 and Cys-133 (Figure

Scheme 1. Structural Formulas of Compounds 1–3.



L-1-*O*-octyl-2-heptylphosphonyl-*sn*-glycero-3-phosphoethanolamine (**1**)



R= -H (**2**)
R= $-C_6H_5$ (**3**, LM-1228)

* Author to whom correspondence should be addressed.
† A preliminary report of this work has been presented at the XIIth Annual Conference of the Molecular Graphics Society, June 7, 1993, Interlaken (Abs. P70).
‡ European Molecular Biology Laboratory.
§ Universidad de Alcalá de Henares.
∥ Laboratorios Menarini S.A.

0022-2623/94/1837-0337$04.50/0