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Stereoselective Binding of an Enantiomeric Pair of Stromelysin-1 Inhibitors Caused by Conformational Entropy Factors

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Abstract—Isothermal titration calorimetry was used to analyze the binding of an enantiomeric pair of inhibitors to the stromelysin-1 catalytic domain. Differences in binding affinity are attributable to different conformational entropy penalties suffered upon binding. Two possible explanations for these differences are proposed.

Introduction

Many chiral drugs exhibit stereoselectivity in their binding affinities.[1–8] In some cases, the reasons for this are straightforward and easily recognized by examining the structures of the complexes. For example, a bulky substituent in the ligand may cause binding of one enantiomer to be less favorable because of steric concerns. However, the reasons for stereoselectivity may be less readily apparent, and in some cases direct structural information about the complexes cannot be obtained. In this report, we demonstrate that a complete thermodynamic analysis of binding, obtained using isothermal titration calorimetry (ITC), can provide important information about the causes of stereoselective binding.

Matrix metalloproteinases (MMPs) play important roles in tissue remodeling and wound healing.[9] However, disruption of the control mechanisms for MMP expression and regulation can contribute to diseases including rheumatoid and osteoarthritis,[10] multiple sclerosis,[11] and tumor metastasis.[12,13] Moreover, it has recently been reported that mice that are deficient in stromelysin-1 (MMP-3) have reduced susceptibility to contact hypersensitivity,[14] raising the possibility that selective inhibitors of stromelysin-1 may be useful for the treatment of allergic reactions.

In a recent report, the binding of three inhibitors to the stromelysin-1 catalytic domain (SCD) was studied by ITC.[15] The thermodynamic profiles obtained in that study provided insights into the types of interactions that participate in binding and the relative magnitudes of their contributions to the overall binding free energy. In this study, we compared the binding thermodynamics of an enantiomeric pair of inhibitors (PD169469 and PD166793; *R*- and *S*-2-[4′-bromo-biphenyl-4-sulfonyl-amino]-3-methyl-butyric acid, respectively) to the SCD. A 9-fold difference in binding affinity between the stereoisomers was observed. Based on the thermodynamic profiles of the binding of these inhibitors (ΔG, ΔH, ΔS, and ΔC_p), we propose that this difference in affinity is due to a difference in the conformational entropy penalties that accompany the binding of the enantiomers. Two possible explanations for these differences are discussed.

Results

The structure of PD169469 is shown in Figure 1 along with its proposed sites of interaction with the

Abbreviations: MMP, matrix metalloproteinase; ITC, isothermal titration calorimetry; SCD, stromelysin-1 catalytic domain; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); MES, 2-(*N*-morpholino)-ethane sulfonic acid.

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enzyme.[16–18] PD169469 has a single chiral center at the point where the P1 isopropyl group is attached and has the *R*-configuration. The thermodynamic parameters for binding of PD169469 to the SCD were determined and compared to those previously obtained for the *S*-isomer, PD166793.[15]

ITC experiments were conducted at 22 °C, pH 6.7 in three different buffers.[19] Binding of carboxylic acid inhibitors to the SCD has been shown to involve the transfer of approximately two protons from the buffer to the complex at pH 6.7, due to protonation of the inhibitor carboxylate group and of histidine 224 of the enzyme.[15] The enthalpy change upon binding of the *R*-isomer varied in a linear fashion with the deprotonation enthalpy of the buffer (data not shown), indicating that proton transfer occurs during binding of this inhibitor as well. The slopes and intercepts of these lines give the number of protons transferred and the corrected ΔH of binding, respectively,[20] which are reported in Table 1 for both enantiomers.

The K_d for the *S*-isomer was 9-fold lower than for *R*, resulting in a ΔG that was 1.3 kcal/mol more negative. However, ΔH was 1.4 kcal/mol less negative for the *S*-isomer than for *R*. The difference in binding affinities is therefore caused by a more negative entropy change upon binding of the *R*-isomer compared to *S* (Table 1).

Binding affinities for stromelysin inhibitors are highly pH-dependent. The K_d for binding of the *R*-isomer at pH 6.0, 22 °C was 54.9 (±19.9) nM, 12-fold lower than the value of 679 (±151) nM determined at pH 6.7. The *S*-isomer showed a similar 17-fold difference in K_d values between pH 6.0 and 6.7 [4.4 (±2.2) nM and 76.6 (±17.7) nM, respectively]. The binding affinity at pH 6.0 was 12-fold higher for the *S*-isomer than for *R*,[†] similar to the 9-fold difference observed at pH 6.7.

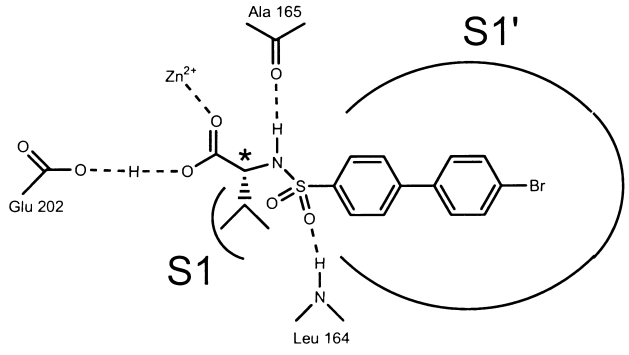

Figure 1. Structure of the stromelysin inhibitors and their proposed sites of interaction with the enzyme.[16–18] The inhibitors contain a single chiral center, denoted by an asterisk, at the point of attachment of the P1 isopropyl substituent. The *R*-isomer (PD169469) is shown; the *S*-isomer is PD166793 (compounds **11c′** and **11c**, respectively, from ref 16).

[†]O'Brien et al.[16] reported IC_{50} values at pH 6.0, 22 °C, of 7 and 8 nM for the *S*- and *R*-isomers, respectively. We are unable to explain the large discrepancy in the apparent degree of stereoselectivity between these IC_{50} values and the K_d values reported here. However, it should be noted that K_d values are more rigorously defined thermodynamic parameters.

The change in heat capacity (ΔC_p; i.e. $d\Delta H/dT$) provides a measure of the degree of solvent expulsion that occurs upon binding,[21] and can provide an estimate of the extent of hydrophobic interactions that are formed in the complex. ITC experiments were performed at three temperatures with the *R*-isomer. The ΔC_p for binding of *R* was approximately 3-fold more negative than the value obtained for *S* (Table 1). This suggests that binding of the *R*-isomer involves a greater degree of burial of hydrophobic surface than is the case for *S*. However, as discussed below, molecular modeling experiments have suggested that this difference in ΔC_p values could also be accounted for by the formation of a water-mediated hydrogen bond upon binding of the *R*-isomer.

Discussion

In a previous study, we used ITC to analyze the binding of a set of inhibitors to the stromelysin-1 catalytic domain.[15] Several conclusions were reached about the types and magnitudes of contributions of different substituents to the overall free energies of binding. The data also suggested that the S1′ subsite of the enzyme undergoes a large decrease in conformational flexibility upon binding of substituents that make substantial interactions with, but do not completely fill, the subsite, leading to an entropic penalty and consequent decrease in binding affinity. This is supported by structural data recently obtained with similar inhibitors.[17,22]

In this study, we compared the thermodynamic parameters for binding of an inhibitor with the *R*-configuration at the P1 substituent (PD169469, Fig. 1) to those obtained for the *S*-isomer (PD166739). At pH 6.7 and 22 °C, the *S*-isomer had a 9-fold higher binding affinity than the *R*-isomer (Table 1). However, the ΔH

Table 1. Thermodynamic parameters for the binding of *R*- and *S*-isomers of a carboxylic acid inhibitor to the stromelysin-1 catalytic domain[a]

Inhibitor	PD169469 (*R*-isomer)[b]	PD166793 (*S*-isomer)[b]
K_d (nM)[c]	679 (151)	76.6 (17.7)
ΔG (kcal/mol)	−8.33 (0.88)	−9.62 (0.13)
ΔH (kcal/mol)[d]	−11.5 (0.2)	−10.1 (0.3)
ΔS (cal/mol K)	−10.6 (3.0)	−1.46 (1.12)
protons transferred[e]	1.79 (0.06)	1.71 (0.07)
ΔC_p (cal/mol K)	−184 (13)	−66.1 (23.4)

[a]All data for the *R*-isomer were obtained at pH 6.7, 22 °C, except for ΔC_p which was obtained over the range of 12–22 °C. The thermodynamic parameters for the *S*-isomer were obtained from ref 15.
[b]Values in parentheses are standard deviations (obtained from the fitting program or calculated as described in ref 32). The average binding stoichiometries were 1.16 (±0.07) and 1.02 (±0.08) for the *R*- and *S*-isomers, respectively.
[c]Average values from titrations carried out in three buffers.
[d]Buffer-corrected values obtained from the *y*-intercepts of plots of $\Delta H_{apparent}$ versus the ΔH of deprotonation for each buffer (not shown). Values for $\Delta H_{deprotonation}$ of the buffers were obtained from ref 33.
[e]Net number of protons transferred to the enzyme/inhibitor complex upon binding, obtained from the slopes of plots of $\Delta H_{apparent}$ versus the ΔH of deprotonation of each buffer.

for binding of *R* was more favorable than for *S*, indicating that the lower binding affinity of *R* is due to a more unfavorable ΔS value.

The ΔC_p for binding of the *R*-isomer was nearly 3-fold more negative than for *S*, suggesting that binding of *R* results in the burial of a larger hydrophobic surface area[23] and a more favorable solvational entropy term for the binding of *R* compared to *S*. The structures of complexes of the SCD with either of these inhibitors are not known. However, molecular modeling experiments have suggested that the isopropyl group at P1 of the *R*-isomer is somewhat more buried in the S1 subsite than is the case for *S*.[16] This could account for some of the difference in buried hydrophobic surface area, but only a small fraction. Even if the P1 isopropyl group were completely buried, and if we assume that the enzyme buries a comparable amount of hydrophobic surface area at the S1 subsite, this interaction would only contribute a maximum of about -51 cal/mol K to the ΔC_p value.[23,24] Therefore, if the additional burial of hydrophobic surface area is responsible for the more negative ΔC_p of the *R*-isomer, part of it must occur elsewhere in the binding site.

One possible explanation for the differences in ΔC_p, ΔH, and ΔS between the two stereoisomers is as follows. The deep S1′ subsite of the SCD undergoes considerable movement upon the binding of inhibitors with P1′ substituents of intermediate size.[17,22] Comparison of the binding thermodynamics of PD166793 with those obtained for an inhibitor with a smaller isobutyl moiety at P1′ suggested that groups of a particular range of sizes are able to trigger this conformational change, while smaller groups cannot.[15] This rigidification of the enzyme contributes to the decrease in conformational entropy that occurs upon binding. The total change in entropy for binding of PD169469 (the *R*-isomer) was more negative than for PD166793 (the *S*-isomer). We propose that the S1′ subsite undergoes a more extensive loss of conformational entropy upon binding to the *R*-isomer than to *S*, and that this contributes to the lower binding affinity of the *R*-isomer. An increase in π-stacking and other van der Waals interactions between the S1′ subsite and the *R*-isomer could account for the more negative ΔH compared to *S*.

If the S1′ subsite forms more extensive hydrophobic interactions with the *R*-isomer than with *S*, this would lead to the expulsion of more bound solvent molecules from the interface between the P1′ moiety and the enzyme. This would be reflected in a more negative ΔC_p value for the *R*-isomer, as we have in fact observed. However, the more negative ΔS value observed for the *R*-isomer is the opposite of what would be expected if increased solvent expulsion accompanying the binding of *R* were the major contributor to the difference in ΔS values. In this case, it appears that the entropic cost of conformational rigidification is greater than the entropic advantage obtained from desolvation at the S1′ subsite.

Another explanation can be proposed for the differences in the binding thermodynamics between the two enantiomers. Molecular modeling experiments suggested that a hydrogen bond between the sulfonamide NH of the *S*-isomer and the backbone carbonyl of alanine 165 might be replaced by a water-mediated 'bridging' hydrogen bond to proline 221 in the *R*-isomer.[16] If this is indeed the case, it could account for some or all of the difference in ΔC_p values between the two isomers. Water-mediated hydrogen bonds can cause the ΔC_p value for a binding interaction to be more negative than would be predicted based on the degree of burial of polar and apolar surface areas. A mutation in DNA gyrase that replaced a direct hydrogen bond with a bridging water molecule led to a ΔC_p for binding that was 159 cal/mol K more negative.[25] This was accompanied by ΔH and ΔS values for binding to the mutant that were both more negative than for wild-type, as was observed for the *R*-stromelysin inhibitor compared to *S*.

The burial and concomitant desolvation of polar and apolar surfaces during protein folding or ligand binding have opposite effects on the sign of ΔC_p, with somewhat larger effects per unit of surface area being observed for apolar surfaces.[23,26] A physical explanation for the opposing effects of dehydration of polar and apolar groups on ΔC_p has recently been presented.[27–29] This interpretation would also explain the unusually negative ΔC_p values observed when water molecules become sequestered in a binding interface, as in the case of water-mediated hydrogen bonds.[25] The sequestered water molecules would be highly constrained by being held tightly in place by the other species of the complex, resulting in their having reduced abilities to fluctuate between energetic states and thus a lower C_p than bulk water. This interaction would also cause a decrease in the conformational entropy and enthalpy of the system.

These findings have implications for the use of molecular modeling in the drug design process. Our results suggest that an inhibitor that fits more snugly into a binding site can have a *lower* binding affinity than one that does not bury as much surface area if a conformational entropy penalty accompanies the formation of these tight contacts. Other factors, such as the trapping of a water molecule into a hydrogen-bonding network, may contribute to a decrease in conformational entropy as well. When computational methods are used to estimate binding affinities, the possibility of extensive conformational changes in either molecule, and the effects these could have on the ΔS of binding, should be taken into account.

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