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Impact of Mobility on Structure-Based Drug Design for the MMPs

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Structural information on protein−ligand complexes is a fundamental necessity for the rational design of new drug candidates (for reviews, see refs 1−4). The beneficial impact of structure-based design efforts is evident from the ongoing success in delivering drugs for clinical evaluation.[1,2,5,6] The general paradigm of a structure-based approach is to utilize all of the available structural information to improve the ligand's affinity by optimizing its fit and interaction with the protein target. An increase in affinity is evaluated on the basis of a number of factors that contribute to the overall binding energy,[7,8] where a variety of computational methods are used to predict the relative improvement in binding affinity.[3,4,8] The computational methods used in typical drug design projects are generally limited in scope because of the large number of potential ligands to evaluate. This combined with limited computer resources and the complexity of the calculations result in the use of modeling approaches that do not typically yield rigorous predictions of binding free energies, where solvent effects and mobility are two factors that typically suffer from approximations. As a result, for a protein family like the matrix metalloproteinases (MMPs), that exhibit an inherent mobility in the active site,[9−13] the accurate modeling of potential inhibitors poses a particular concern. This problem was clearly illustrated in recent MMP X-ray structures that demonstrated the ability of side chains in the active site to undergo conformational changes to accommodate a bound inhibitor that was not readily apparent in prior structures.[12,14] Our effort further illustrates the contribution of dynamics to inhibitor binding from the NMR analysis of MMP complexes.

The design of inhibitors of various MMPs for use as therapeutic agents in the treatment of arthritis and cancer has been an exceptionally active area of research.[14,15] The MMPs are involved in the degradation of the extracellular matrix that is associated with normal tissue remodeling, and as result, MMP expression and activity is highly controlled. The apparent loss in this regulation can result in the pathological destruction of connective tissue and an ensuing disease state. The MMP family consists of more than 25 enzymes, and it has been postulated that the toxicity demonstrated by many MMP inhibitors in clinical trials may result from nonspecific inhibition. Thus, the current approach relies on structure-based design of inhibitors of specific MMPs, where selectivity against MMP-1 may be a desirable trait.

The extensive structural data available for the MMPs[14] has enabled the identification of an obvious approach for designing specificity by taking advantage of the sequence difference and distinct size and shape of the S1′ pocket. A number of examples have been previously reported using this approach.[11,12,16,17] Nevertheless, the observed mobility of the MMP active site may complicate the design of potentially selective inhibitors.[9−13]

On the basis of the structural information available at the time, a series of hydroxamic acid compounds incorporating a butynyl P1′ group was expected to be selective against MMP-1 activity on the basis of a poor fit in the MMP-1 S1′ pocket (Figure 1). While a majority of the designed compounds did exhibit selectivity, surprisingly some compounds were shown to bind well to MMP-1 and other MMPs in an IC_{50} range of 5−40 nM. This better than expected binding is attributed to some interesting dynamics present in the NMR structures of **1** bound to MMP-1 (IC_{50} 40 nM) and MMP-13 (IC_{50} 5 nM). It is also consistent with the MMP active-site elasticity previously observed.[12,14]

1 exhibits a slow exchange between two distinct conformations when bound to MMP-1. This is indicated by the observation of two sets of NMR assignments for **1** in the MMP-1 complex (Figure 2). The purity and the presence of a single conformation for free **1** in 100% DMSO was verified by the observation of a single set of NMR resonances. The slow-exchange conformations for **1** is apparent in both the 2D-^{12}C,^{12}C-filtered NOESY, and the 3D ^{13}C-edited/^{12}C-filtered NOESY experiments where a distinct set of intra- and interresidue NOEs are observed between the two different slow-exchanging conformers and MMP-1. The two distinct conformers exist at an approximate 1:2 ratio where the structural difference between the conformers is subtle. The conformers are differentiated by the relative orientation of the isopropyl group of **1**. In one conformer, both isopropyl methyls interact with both N80 and H83, while in the other conformer only one methyl interacts with N80 while the other interacts with H128. Also, a different set of NOEs is observed from the aromatic ring in **1** to the MMP-1 dynamic active-site loop (residues 138−144) for the two conformers. This also suggests that the conformation of the MMP-1 dynamic loop is distinct between the two conformers. Further supporting the contribution of dynamics to the affinity of **1** with MMP-1 is the observation that only one conformation for the isopropyl group is present for **1** complexed with MMP-13, as evident from a single set of NMR resonances.

In addition to the slow-exchange conformations, **1** is also in fast exchange between two bound conformations. This is indicated by the fact that the compound exhibits NOEs to two distinct sites on the protein where it is not possible for the binding interactions to occur simultaneously. The two fast-exchange conformers differ in the relative orientation of the butynyl group (Figure 3). For one conformer, the butynyl tail binds toward the center of the dynamic loop and exhibits NOEs to Y137 and L135. In the second fast-exchange conformer, the butynyl tail is parallel to helix α_2 and the dynamic loop and has NOEs to residues V115 and L81. It is important to note that helix α_2 incurs significant chemical shift changes upon binding **1** that has not been seen with other classes

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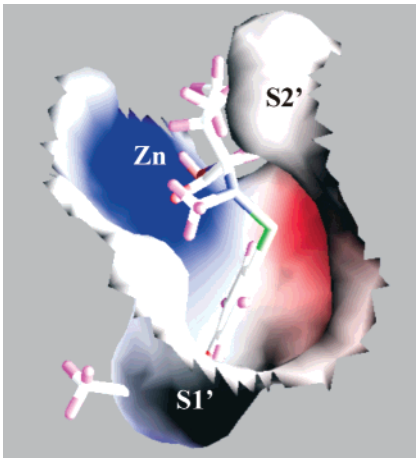

Figure 1. **1** (IC_{50} 40 nM) positioned in the S1′ binding pocket of free MMP-1 illustrating the potential steric clash upon binding of the compound with MMP-1.

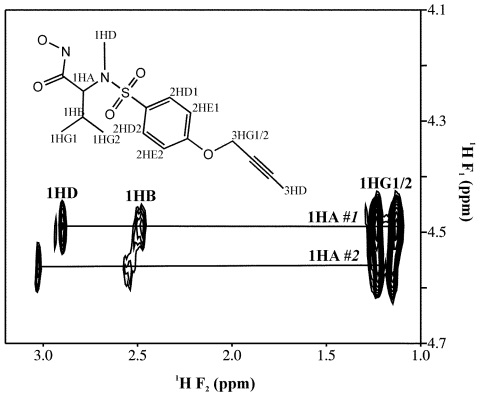

Figure 2. Expanded region of the 2D-^{12}C,^{12}C-filtered NOESY experiment for **1** complexed to MMP-1. The two slow-exchanging conformations for **1** are evident by the two distinct sets of NMR resonances, which are unique from the free assignments for **1**. The labeled chemical structure for **1** is also shown.

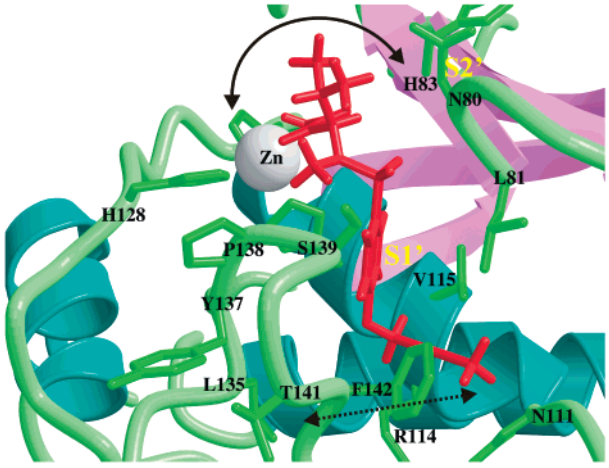

Figure 3. Ribbon diagram of one-potential conformation of the MMP-1:**1** complex, where the solid arrow indicates the rocking motion associated with the slow-exchange and the dashed arrow indicates the fast-exchange "twist" motion of the butynyl group pocket. Side chains (green) for residues involved in the interaction with **1** (red) are shown and labeled.

of inhibitors. The two fast-exchange conformers are also present in the MMP-13:**1** complex, where the same relative residues are involved in the interaction with the butynyl methyl.

The predicted low affinity of **1** with MMP-1 based on a static model from the original "closed-form" of MMP-1 was clearly misleading, and the resulting structure for the complex emphasizes the contribution of dynamics to the binding energy of MMP inhibitors. Recent modeling efforts with the "open-form" suggests that **1** is capable of binding MMP-1. Thus, the binding of **1** to MMP-1 overcomes the steric clash and poor fit of the butynyl group in the MMP-1 S1′ pocket by maintaining a significant entropic contribution to its free energy of binding and through the elastic nature of the MMP active-site. This is accomplished by a rapid twisting motion of the butynyl group between two reasonable binding modes in the S1′ pocket, an apparent slow "rocking" motion of the isopropyl group about the catalytic Zn, and the active-site loop and side-chain motions observed in prior structures.[9−13] Both sets of compound motions maintain favorable enthalpic interactions that are interchanged between the different conformers. In effect, the intrinsic energetic cost of opening the S1′ pocket to accommodate **1** is partially compensated for by the motions exhibited by the compound in the complex. The process of compensating for poor steric interactions by mobility is a delicate balancing act. An analogue of **1** where the isopropyl group is removed shows diminished binding to both MMP-1 (IC_{50} 2 μM) and MMP-13 (IC_{50} 100 nM). The elimination of the isopropyl group removes a number of beneficial interactions in the S2′ pocket that are present in either of the slow-exchanging MMP-1 conformers. More importantly, the presence of the isopropyl group effectively buries and shields the hydroxamic acid from the solvent upon chelating the active-site Zn, significantly increasing the stability of this interaction.[18] Clearly, just the presence of motion is not sufficient to compensate for the poor fit in the S1′ pocket and the resulting energetic cost to open the binding site, since other favorable enthalpic interactions are also required. Nevertheless, understanding the inherent mobility of both the ligand and protein is a valuable asset in aiding the drug design process, where NMR plays a unique role in obtaining this information.

Supporting Information Available: Experimental procedures, NMR resonances, and NOE assignments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Supplement Information

Experimental Procedures

I. NMR Sample Preparation.

Uniformly (>95%) ^{15}N- and $^{15}N/^{13}C$-labeled human recombinant MMP-1 was expressed in *E. coli* and purified as described previously[1,2] except that anion exchange was carried out on Source 30Q anion exchange resin (Pharmacia, Piscataway, NJ).

The MMP-1:WAY-171230 NMR sample contained 1mM ^{15}N- or $^{15}N/^{13}C$-labeled MMP-1 with WAY-171230 in a 1:1 ratio. The sample was prepared by repeated buffer exchange using 20-30ml solution containing 10mM deuterated Tris-Base, 100mM NaCl, 5mM $CaCl_2$, 0.1mM $ZnCl_2$, 2mM NaN_3, 10mM deuterated DTT, and 0.2mM WAY-171230 in either 90% H_2O/10 % D_2O or 100% D_2O. Excess WAY-171230 was removed by additional buffer exchanges where WAY-171230 was not present in the buffer.

II. NMR Data Collection.

All spectra were recorded at 35° C on a Bruker AMX600 spectrometer using a gradient enhanced triple-resonance $^1H/^{13}C/^{15}N$ probe.

The assignments of the ^1H, ^{15}N, and ^{13}C resonances of MMP-1 in the MMP-1:WAY-171230 complex were based on a minimal set of experiments as previously described[3]. The acquisition parameters for each of the experiments used in determining the solution structure of the MMP-1:WAY-171230 complex were as reported previously[4].

The resonance assignments and bound conformation of WAY-171230 in the MMP-1: WAY-171230 complex were based on the 2D $^{12}C/^{12}C$-filtered NOESY[5,6].

The MMP1:WAY-171230 structure is based on observed NOEs from the 3D ^{15}N-edited NOESY[7,8], 3D ^{13}C-edited/^{12}C-filtered NOESY[9] and $^3J_{NH\alpha}$ coupling constants measured from the relative intensity of Hα crosspeaks to the NH diagonal in the HNHA experiment[10]. The 3D ^{15}N-edited NOESY and 3D ^{13}C-edited/^{12}C-filtered NOESY experiments were collected with 100 msec and 110 msec mixing times, respectively. The acquisition parameters for all experiments for MMP-1 with WAY-171230 were identical to parameters reported previously for free MMP-1[11].

III. Structure Calculations.

The NOEs assigned from 3D ^{13}C-edited/^{12}C-filtered NOESY and 3D ^{15}N-edited NOESY experiments were classified into strong, medium or weak[12,13]. Upper distance limits for distances involving methyl protons and non-stereospecifically assigned methylene protons were corrected appropriately for center averaging[14].

The structures were calculated using the hybrid distance geometry-dynamical simulated annealing method of Nilges et al. (1988)[15] with minor modifications[16] using the program XPLOR[17], adapted to incorporate pseudopotentials for $^3J_{NH\alpha}$ coupling constants[18], secondary $^{13}C\alpha/^{13}C\beta$ chemical shift restraints[19] and a conformational database potential[20,21].

The restraints used for the refinement of the inhibitor-free MMP-1 NMR structure[11] were amended with the distance restraints observed between MMP-1 and WAY-171230 from the 3D ^{13}C-edited/^{12}C-filtered NOESY and 3D ^{15}N-edited NOESY experiments and intramolecular restraints observed for WAY-171230 from the 2D ^{12}C-filtered NOESY experiment. Four separate NOE constraint sets were used corresponding to the four pairs of slow-exchanging and fast-exchanging conformers. The inhibitor-free MMP-1 NMR restraints were modified as appropriate for residues in the vicinity of the active site (80-83, 114-119 and 136-142) by either removing restraints inconsistent with the MMP-1:WAY-171230 structure and/or by the addition of new restraints observed in the complex. Additionally, the MMP-1:WAY-171230 complex was refined using the $^3J_{NH\alpha}$ coupling constants determined from the HNHA[10] experiment and secondary $^{13}C\alpha/^{13}C\beta$ chemical shift restraints from the assignments for the complex. The bound conformation for WAY-171230, the starting structure and refinement of MMP-1:WAY-171230 structure were determined as described previously[3].

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Supplement Tables

Table 1 Supplement:^{1}H NMR (ppm) assignments for compound **1**. Conformation #1 is the Major form in MMP-1.

Compound 1	Free	MMP-1 #1	MMP-1 #2	MMP-13
1HG1	0.80	1.13	1.15	1.17
1HG2	0.83	1.24	1.23	1.26
1HB	2.29	2.49	2.55	2.46
1HA	3.74	4.49	4.56	4.26
1HD	2.82	2.90	3.02	2.86
2HD1/2	7.73	7.71	7.93	7.67
2HE1/2	7.12	7.26	7.35	7.15
3HG1/3HG2	4.86	5.22	-	5.00
3HD	1.86	2.34	2.34	2.21

Table 2 Supplement: Observed NOEs to compound **1** that differentiates the two fast-exchange conformations.

MMP-1 Fast-exchange conformation #1	MMP-1 Fast-exchange Conformation #2
NOEs to 3HD	NOEs to 3HD
3HE1/2	R114 δ
L81 δ	L135 α
N111 HN	L135 δ
R114 δ	Y137 HN
V115 γ	F142 α
F142 α	F142 β
F142 β	
F142 δ	

Table 3 Supplement: Observed NOEs to compound **1** that differentiates the two slow-exchange conformations, where conformation #1 is the major form.

MMP-1 Slow-exchange conformation # 1			MMP-1 Slow-exchange conformation #2		
NOEs to 1HG1	*NOEs to1 HG2*	*NOEs to 2HD1/2, 2HE1/2*	*NOEs to1 HG1*	*NOEs to 1HG2*	*NOEs to 2HD1/2, 2HE1/2*
H128 ε	N80 α	L81 δ	H83 α	N80 α	L81 δ
	N80 β	A82 β	H83 β	N80 β	V115 γ
	L81 HN	V115 α		L81 HN	S139 α
	A82 HN	V115 γ		A82 HN	T141 α
		P138 α			T141 γ
		S139 α			F142 β
		F142 α			
		F142 β			
		F142 δ			

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Supplement Tables

Table 1 Supplement: ^{1}H NMR (ppm) assignments for compound **1**. Conformation #1 is the Major form in MMP-1.

Compound **1**	Free	MMP-1 #1	MMP-1 #2	MMP-13
1HG1	0.80	1.13	1.15	1.17
1HG2	0.83	1.24	1.23	1.26
1HB	2.29	2.49	2.55	2.46
1HA	3.74	4.49	4.56	4.26
1HD	2.82	2.90	3.02	2.86
2HD1/2	7.73	7.71	7.93	7.67
2HE1/2	7.12	7.26	7.35	7.15
3HG1/3HG2	4.86	5.22	-	5.00
3HD	1.86	2.34	2.34	2.21

Table 2 Supplement: Observed NOEs to compound **1** that differentiates the two fast-exchange conformations.

MMP-1 Fast-exchange conformation #1	MMP-1 Fast-exchange Conformation #2
NOEs to 3HD	NOEs to 3HD
3HE1/2	R114 δ
L81 δ	L135 α
N111 HN	L135 δ
R114 δ	Y137 HN
V115 γ	F142 α
F142 α	F142 β
F142 β	
F142 δ	

Table 3 Supplement: Observed NOEs to compound **1** that differentiates the two slow-exchange conformations, where conformation #1 is the major form.

MMP-1 Slow-exchange conformation # 1			MMP-1 Slow-exchange conformation #2		
NOEs to 1HG1	*NOEs to1 HG2*	*NOEs to 2HD1/2, 2HE1/2*	*NOEs to1 HG1*	*NOEs to 1HG2*	*NOEs to 2HD1/2, 2HE1/2*
H128 ε	N80 α	L81 δ	H83 α	N80 α	L81 δ
	N80 β	A82 β	H83 β	N80 β	V115 γ
	L81 HN	V115 α		L81 HN	S139 α
	A82 HN	V115 γ		A82 HN	T141 α
		P138 α			T141 γ
		S139 α			F142 β
		F142 α			
		F142 β			
		F142 δ			

ABSTRACT Structure-based approaches for drug design generally do not incorporate solvent effects and dynamic information to predict inhibitor-binding affinity because of practical limitations. The MMPs have previously been demonstrated to exhibit significant mobility in their active sites. This dynamic characteristic significantly complicates the drug design process based on static structures, which was clearly observed for a class of hydroxamic acids containing a butynyl moiety. Compound **1** was expected to be selective against MMP-1 based on predicted steric clashes between the butynyl P1' group and the S1' pocket, but the observation of complex inhibitor dynamics in the NMR structure of MMP-1:compound **1** provides an explanation for the low nanomolar binding to MMP-1.