BINDING MECHANISMS OF THROMBIN INHIBITORS AND TRYPSIN INHIBITORS REVEALED BY X-RAY CRYSTALLOGRAPHY

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Abstract The reason for the difference between the binding mechanisms of an anti-thrombosis drug, Argatroban ((2R,4R)-4-methyl-1-[N²-((RS)-3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidine carboxylic acid, MQPA), and bovine basic pancreatic trypsin inhibitor (BPTI) was clarified by X-ray crystallographic analysis of MQP-trypsin complex: The difference is not due to the carboxyl group in Argatroban, but is attributed to the total conformation of the inhibitor. The binding mechanism explains the specificities of a series of Argatroban-related inhibitors against serine proteases. The binding structure of an anti-pancreatitis drug, Nafamostat, to trypsin unexpectedly showed that the amidinonaphthalene portion was inserted into the specificity pocket. Another anti-pancreatitis drug, Camostat, was found to bind covalently to trypsin. These results provide bases for novel drug design strategies.

Key words binding mechanism, inhibitor, thrombin, trypsin, X-ray crystallography

INTRODUCTION

The three-dimensional structures of drug-receptor complexes are essential to comprehend drug action mechanisms. We have determined a number of inhibitor-enzyme complex structures by X-ray crystallography, which will support the rational molecular design of novel drugs. An example of our studies was about an anti-thrombosis drug, Argatroban ((2R,4R)-4-methyl-1-[N²-((RS)-3-methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidine carboxylic acid, MQPA) [1]. The binding nature of Argatroban was further elucidated by a study of the complex formed between MQP (an Argatroban derivative lacking the carboxyl group) and trypsin. From these studies we could get an insight into the specificities of a series of Argatroban-related inhibitors against serine proteases including trypsin, thrombin, plasmin and factor X_a. The binding mechanisms of anti-pancreatitis drugs, Nafamostat and Camostat, were also investigated for deeper understanding of the binding mechanism.

MATERIALS AND METHODS

The inhibitor-enzyme complexes were crystallized by a hanging drop method. TABLE 1 shows crystallization conditions. Summaries of data collection, crystallographic data and structural refinement are given in TABLES 2 through 4.

TABLE 1 Crystallization Conditions

	MQP	Nafamostat	Camostat
Protein Solution (per ml)			
Trypsin (Bovine Pancreatic)	90 mg	60 mg	64 mg
Inhibitor	2.5 mg	0.5 mg	4.3 mg
CaCl ₂	1.0 mg	1.0 mg	0.3 mg
Ammonium Sulfate	8.6 mg	5.3 mg	2.8 mg
Methanol	43 μ l	-	90 μ l
Reservoir Solution			
Ammonium Sulfate	1.0 M	1.5 M	1.15 M
Crystal Dimension (mm ³)	0.6x0.5x0.4	1.0x0.3x0.2	0.8x0.3x0.1

TABLE 2 Summary of Data Collection

	MQP	Nafamostat	Camostat
Diffractometer	MAC Science DIP-300	Enraf-Nonius CAD4	Rigaku RAXIS-IIC
No. of Reflections	12782	10316	15695
Resolution (Å)	2.07	2.5	2.0
R _{merge} (%)	4.0	-	7.6

TABLE 3 Summary of Crystallographic Data

		MQP	Nafamostat	Camostat
Cell Parameters (Å)	a	55.46	63.25	63.82
	b	56.67	69.11	69.05
	c	66.88	63.72	63.54
Space Group		P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Z		4	4	4

TABLE 4 Summary of Refinement

	MQP	Nafamostat	Camostat
Resolution (Å)	7.0 - 2.1	7.0 - 2.5	7.0 - 2.0
Number of Reflections	11622 (>3 σ)	7856 (>1 σ)	15150 (>2 σ)
R Factor (%)	17.8	17.7	18.0
R.M.S. Bond Length (Å)	0.017	0.015	0.017
R.M.S. Angle Distance ^a (Å)	0.050	0.033	0.035

^a Distance between two atoms separated by two bonds.

RESULTS AND DISCUSSION

Binding mechanism of Argatroban Bovine basic pancreatic trypsin inhibitor (BPTI) had been thought to be the general basis for drug design against serine proteases[2]. By X-ray crystallographic study, however, we found that the binding mechanism of a thrombin inhibitor, Argatroban, to trypsin was different from that of BPTI[1]: Argatroban forms (1) hydrogen bonds with the main chain N-H and C=O groups of Gly216, (2) acid-base hydrogen bonds at the bottom of the specificity pocket and (3) a hydrogen bond through a water molecule to the oxyanion hole (the main chain N-H groups of Gly193 and Ser195), whereas BPTI utilizes the hydrogen bonds of (2) and (3). If the carboxyl group of Argatroban, which is more likely to form a hydrogen bond to the oxyanion hole than the carbonyl group, were responsible for the difference, an Argatroban derivative without the carboxyl group, MQP, would take a BPTI-type interaction with the enzyme.

The X-ray analysis of MQP-trypsin complex, however, revealed that the derivative bound in basically the same way as Argatroban (Fig. 1). The interactions (1) and (2) were found also in this complex, as is schematically depicted in Fig. 2. The fact that MQP takes the same binding pattern notwithstanding the lack of the interaction (3) suggests that the difference between Argatroban and BPTI binding mechanisms is not due to the carboxyl group in Argatroban. Moreover, our crystallographic data of unbound Argatroban showed that the conformation of Argatroban bound to trypsin was quite the same as that in the unbound state. The difference, therefore, should be attributed to the total conformation of the inhibitor. The derivative MQP, however, is slightly shifted away from the oxyanion hole compared with Argatroban, because the former does not have the interaction (3) which keeps it in contact with the oxyanion hole through a water molecule. Into the gap between the derivative and the oxyanion hole inserted is a sulfate ion instead of a water molecule. These binding structures

consistently account for the fact that the trypsin inhibitory constant K_i of MQP is bigger by one order than that of Argatroban. Specificities of Argatroban-related inhibitors The binding mechanism of Argatroban- and MQP-trypsin complexes explains the binding specificities of a series of Argatroban-related inhibitors against serine proteases including trypsin, thrombin, plasmin and factor X_a. A crystallographic study on thrombin has recently been reported by Bode et al.[5] The thrombin structure shows a large protrusion over His57 portion and an overhang around Trp215. These regions seem to provide the enzyme with additional small cavities to accommodate the two methyl groups of Argatroban. However, if the 4-methyl group of piperidine ring is replaced with a phenyl group, the activity against thrombin is expected to be drastically reduced because of bump against the protrusion, while such a bump is not expected to occur for trypsin. This inference is consistent with experimental data[2]. Argatroban has R chirality at 2- and 4-positions of the piperidine ring, (2R,4R) for short. A (2R,4S) isomer shows weaker inhibitory activity: The K_i values of (2R,4R) and (2R,4S) isomers are 0.019 μM and 0.24 μM for thrombin, and 5.0 μM and 30. μM for trypsin, respectively[3,4]. The reason is readily understood from the stereochemical viewpoint: If the (2R,4S) isomer directs the carboxyl group toward the oxyanion hole, the 4-methyl group bumps against the 'wall' of His57.

Fig. 1 Stereoscopic view of the binding structure of MQP (thick line) in comparison with Argatroban (thin line). The sulfate ion (see text) was omitted for simplicity's sake.

Fig. 2 The binding mechanisms of MQP (right) and Argatroban (left).

Binding mechanism of Nafamostat The binding structure of an anti-pancreatitis drug, Nafamostat, to trypsin is another example of new binding mechanisms. The inhibitor had been expected to insert the guanidinobenzene portion into the specificity pocket, because the derivatives having the functional group in common showed similarly strong activities. The present study, however, revealed that the amidinonaphthalene portion was anchored in the binding pocket, while the guanidinobenzene ring was located in the proximity of His57 and Ser195 (Fig. 3). The result implies that we should modify the guanidinobenzene portion to improve the inhibitory activity rather than the amidinonaphthalene group.

Binding mechanism of Camostat The binding structure of Camostat to trypsin is different from that of Nafamostat in spite of their similar biological activities. p-Guanidinobenzoate portion is inserted into the specificity pocket with a covalent link to Ser195 O^γ while the other portion is released from the complex as is depicted in Fig. 4. The result provides another type of a strategy to novel drug design.

Fig. 3 The binding structure of Nafamostat.

Fig. 4 The binding structure of Camostat.

CONCLUSION

The elucidated three-dimensional structures helped us clearly understand the mechanisms of the drug actions. The accumulating structural information based upon crystallographic analyses will further open up our scope of developing novel drugs.

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