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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6212–6215
Bioorganic & Medicinal Chemistry Letters

Geometry of GPPE binding to picrate and to the urokinase type plasminogen activator

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Received 20 July 2007; revised 31 August 2007; accepted 5 September 2007
Available online 8 September 2007

Abstract—Crystal structure of 2-(4-guanidynephenyl)-1-phenyl-ethanone (GPPE) in two different environments was determined in order to compare the binding geometry of these compound to a simple picrate anion and to protein, urokinase-type plasminogen activator (uPA), which may be treated as a target for anti-cancer drugs. It was shown that the conformation and the hydrogen-bonding formation by GPPE molecule are similar in both environments, but several important differences were discovered and described.

Urokinase (uPA) is a serine proteinase secreted in cells as zymogen (pro-uPA), which after activation forms a two-chain active molecule (sc-uPA). This active molecule can convert the zymogen plasminogen to the active plasmin, which is a protein with broad substrate specificity and is involved in fibrinolysis, tissue remodelling, angiogenesis, embryogenesis, pathogen and tumour cell invasion, and also in metastasis.[1–3] uPA (urokinase-type plasminogen activator) has restricted substrate specificity. Based on its proteolytic capacity, uPA is thought to be an important target for anti-cancer drugs.

The uPA system contains serine proteinase (uPA), two inhibitors (PAI-1, PAI-2) and the membrane linked receptor (uPAR). uPA consists of N-terminal chain with a growth factor domain, a kringle domain and C-terminal region with a serine proteinase domain, which contains the catalytic site. The growth factor domain is involved in the binding to uPA receptor. Activation of uPA depends on its binding to uPAR. uPAR is a glycoprotein and forms three homologous domains (known as D1, D2 and D3), which are linked to the cell membrane by a glycosyl phosphatidylinositol anchor. The complex uPA–uPAR stimulates cell proliferation, enhances cell migration and modulates cell adhesion.[4] A recent study shows that this complex also leads to signal transduction.[5] Active uPA bound to the receptor is inhibited by PAI-1 and PAI-2. Both inhibitors of uPA belong to the serpin family of the proteinase inhibitors. PAI-1 is a single-chain glycoprotein, which reacts rapidly with uPA forming a stable complex. PAI-2 exists in two forms: a nonglycosylated intracellular form and a glycosylated extracellular form. PAI-2 inhibits uPA more slowly than PAI-1[6].

The uPA system is involved in a variety of cell functions: extracellular proteolysis, adhesion, proliferation, chemotaxis, as well as in cancer invasion and metastasis.[7,8] Since uPA system is present in tumour cell environments, it serves as a useful source of tumour markers. The higher levels of uPA, uPAR and PAI-1 are correlated with poor prognosis, whereas the increased level of PAI-2 predicts a favorable outcome in many cancers.[8] Based on these studies the components of uPA-system are attractive targets for anti-cancer therapy. There are several potential ways to influence the uPA system. One of them is the reduction of proteolytic activity of uPA by small synthetic molecule inhibitors. uPA is a trypsin-like serine proteinase, for which the S1 site is a deep pocket with Asp189 residue, engaged in a hydrogen-bonded salt-bridge with the basic side chain of arginine. Most of the uPA inhibitors include in their scaffold an S1-binding element: an aromatic moiety substituted by an amidine or guanidine group. The sequence and structure of trypsin-like serine proteinases are similar at S1, therefore it is difficult to find a specific inhibitor

Keywords: Crystal structure; Urokinase; Inhibitor; Picrate; X-ray diffraction.
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doi:10.1016/j.bmcl.2007.09.020

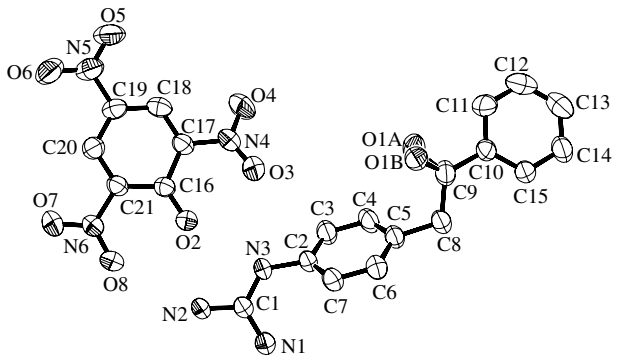

Figure 1. Asymmetric unit of GPPE picrate unit cell together with atom numbering. Hydrogen atoms were omitted for clarity. Atomic displacement ellipsoids are drawn with 40% probability.

only for one of the above-mentioned proteins. The number of known inhibitors of uPA is not small, but only few of them selectively block uPA.[9–13] The crystal structures of several of these inhibitors in complex with uPA, as well as in another environments, have been extensively studied.[14–20]

In this paper, we describe two crystal structures of 2-(4-guanidynephenyl)-1-phenyl-ethanone (GPPE).[21] This compound comprises guanidine group that seems to have higher selectivity towards uPA than the amidine group. The hydrogen-bond formation with different proton acceptors and donors may influence the conformation of the GPPE molecule. In order to examine the effect of different crystalline environments on the conformation of GPPE, we prepared its new crystalline salt, that is, picrate, and also the crystalline complex of GPPE with the catalytic domain of uPA. The preparation details and the experimental crystallographic data of both compounds are given in the Supplementary material.

The projection of GPPE picrate with its atom numbering is shown in Figure 1. GPPE is a molecule containing phenylguanidine and benzoyl moiety. The asymmetric unit consists of one molecule of the GPPE cation and a picrate anion. The molecule of GPPE forms complex 1:1 with picrate ion via hydrogen bonds.

The protonation of the guanidine group is suggested by the bond lengths of C1–N1, C1–N2 and C1–N3, which are 1.318(2) Å, 1.324(2) Å and 1.333(2) Å, respectively. The angle between the guanidine and benzene planes is 59.30° and the bond length between this groups is 1.433(2) Å. A similar conformation of the phenylguanidinium cation was observed in the structure of bis(phenylguanidinium)carbonate monohydrate,[22] in which there are two independent phenylguanidinium cations. The torsion angle C1–N3–C2–C7 in our structure is 50.1(3)°, whereas in the bis(phenylguanidinium)carbonate monohydrate this angle adopts two different values: 54.4(2)° and 79.7(2)°. In another known structure of bisphenylguanidinium squarate this torsion angle is equal to 43.1°.[23] These observations suggest certain influence of the environment on the conformation of the phenylguanidine.

The guanidine group is almost coplanar with the second benzene ring of GPPE as well as with the benzene ring of the picrate ions. The corresponding angles between the planes are 7.99(14)° and 5.45(13)°, respectively.

The displacement parameters of O1 belonging to the carbonyl group indicate its possible disorder, so this atom was refined in two positions, A and B, resulting in occupation factors of 0.6 and 0.4.

The conformation adopted by the picrate anion in our structure is comparable with those observed in other known structures.[19,24]

The packing of the molecules in the unit cell can be characterized by intermolecular hydrogen bonds. The hydrogen atoms, H2B and HN3, of the nitrogens N2 and N3 of the guanidine group are engaged in two kinds of intermolecular hydrogen bonds. Both protons are donated to the oxygen, O2, of the phenolate leading to formation of the canonical salt bridge. H2B and HN3 are additionally shared by the oxygen atoms of the *ortho*-nitro groups, that is, by O8 and O3, respectively. Thus, the hydrogen bonds, in which H2B and HN3 take part, may be treated as bifurcated. The second hydrogen atom, H2A of the nitrogen N2, as well as the hydrogen atom H1B of the third nitrogen N1, are donated to the oxygen atoms, O8 and O7, of the *ortho*-nitro group of the picrate anion transformed by the inversion centre with coordinates 1.0, 0.5 and 0.5. In contrast, the second hydrogen atom, H1A, of the nitrogen N1 is involved in the hydrogen bond with O1 of the carbonyl group of another molecule translated by **a**. The described hydrogen bonds together with the donor–acceptor distances are shown in Figure 3a, while their all parameters are given in Table 1.

The picrate anion, the guanidinium cation and the benzoyl group are almost coplanar and form the parallel

Table 1. Parameters of hydrogen bonds in GPPE picrate crystal

D–H	*d*(D–H) (Å)	*d*(H···A) (Å)	<DHA (°)	*d*(D···A) (Å)	A	Symmetry codes
N1–H1A	0.851	2.093	154.02	2.882(12)	O1A	$x+1, y, z$
N1–H1A	0.851	2.141	146.92	2.892(16)	O1B	$x+1, y, z$
N3–HN3	0.910	1.981	151.81	2.816(2)	O2	
N3–HN3	0.910	2.421	135.11	3.133(2)	O3	
N1–H1B	0.953	2.220	159.80	3.132(2)	O7	$-x+2, -y+1, -z+1$
N2–H2A	0.870	2.146	158.50	2.973(2)	O8	$-x+2, -y+1, -z+1$
N2–H2B	0.894	1.943	151.09	2.760(2)	O2	
N2–H2B	0.894	2.422	136.26	3.129(2)	O8	

layers with the distance ca. 3.3 Å. The benzene ring, which contains the guanidine substituent, is situated between these layers and parallel to **bc**.

The binding mode of GPPE to the active site of uPA is shown in Figure 2. The phenylguanidine moiety of the inhibitor occupies S1 specificity pocket and forms canonical salt bridge to the carboxylate of Asp189. The guanidine group is additionally hydrogen-bonded with both oxygen atoms of Ser190, with the carbonyl oxygen of Gly219, as well as with one molecule of water. It is possible that the longer hydrogen bond between N1 of GPPE and the carbonyl oxygen of Ser190 has rather the character of a polar contact. This group is significantly rotated out of the phenyl plane allowing optimal hydrogen bond formation to the Ser190. The carbonyl oxygen binds into the oxyanion hole forming the hydrogen bond with nitrogen atom of Gly193. The described hydrogen bonds together with the donor–acceptor distances are shown in Figure 3b. The hydroxyl group of Ser195 is turned towards the oxyanion hole, which is not occupied by a sulfate anion from the solvent but by water molecule. In other structures with benzamidine moiety we observed a sulfate anion in the oxyanion hole.[14,17–19] The phenyl group is almost parallel to the imidazole ring of His57 and makes also hydrophobic contacts with His99.

The binding mode of GPPE in complex with uPA exhibits several differences in comparison to that of picrate. In none of these conformations the benzene ring and its substituent, guanidyl group, are coplanar, but in the picrate structure the angle between their planes is greater. In the protein environment the phenylguanidine moiety has less room in the S1 pocket. All three nitrogen atoms are involved in hydrogen bonds. Comparison of the hydrogen-bonding patterns in the structure of GPPE picrate and in the structure of GPPE in its complex with uPA is shown in Figure 3. The overall hydrogen-bonding systems are similar, however, they differ in the way of the salt bridge formation. In the picrate structure the Nα (relative to the phenyl) and Nγ are involved in these bonds, whereas in the structure with uPA both Nγ atoms, but not Nα, take part in them. The benzoil

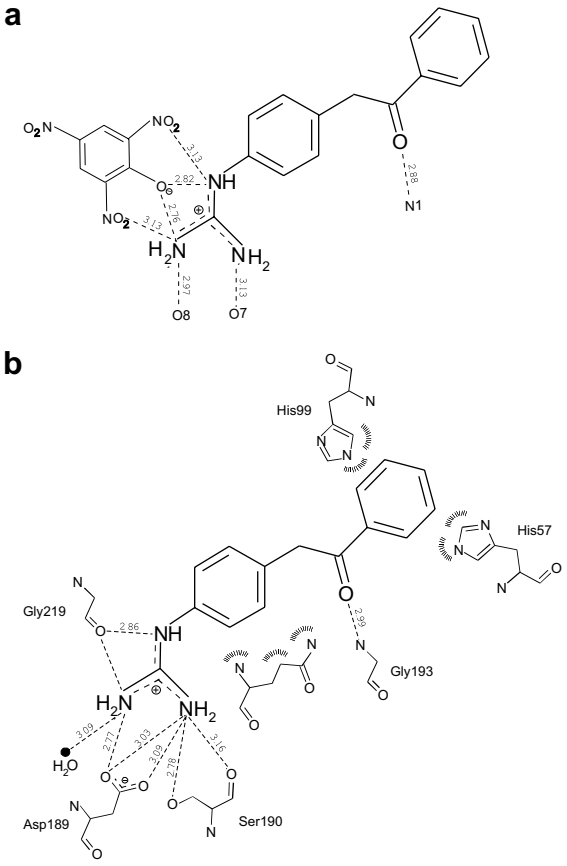

Figure 3. Patterns of hydrogen bonds formed by GPPE molecule in the structures of: (a) picrate. (b) βc-uPA complex. The hydrogen bonds are shown with broken lines with the distances of the interacting atoms given in Å.

groups of GPPE in both structures are mutually almost perpendicular. The orientation of this moiety seems to depend on the carbonyl group that is involved in the hydrogen bond.

The structure of GPPE in complex with another trypsin-like serine proteinase is not yet known. GPPE is almost a fivefold better inhibitor of uPA (K_i = 34 μM)[25] in com-

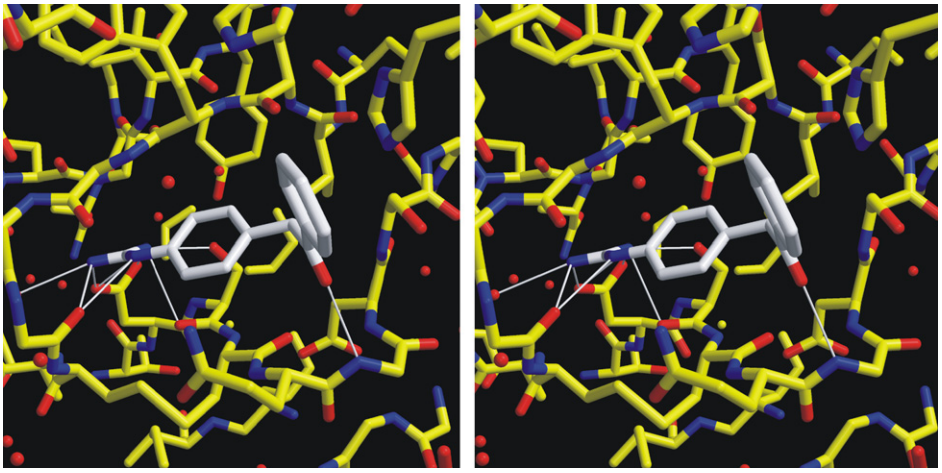

Figure 2. Stereo view of GPPE (white) in the active site of βc-uPA (yellow). The hydrogen bonds are shown with thin lines.

parison to trypsin (K_i = 160 μM) and is a weak inhibitor of tPA (K_i > 1000 μM). In the structure of GPPE with uPA we observed the hydrogen bond between the hydroxyl group of Ser190 and the nitrogen atom of the guanidinium group. This short hydrogen bond (2.78 Å) is not possible in tPA, which contains an alanine in position 190. Therefore, the still weaker interaction of GPPE with tPA seems to be caused by the lack of the hydrogen bond between Ala190 and the inhibitor. Trypsin has also Ser190 and GPPE is a better inhibitor of this enzyme than tPA. The rather good selectivity and binding of GPPE to uPA seems to depend on the guanidine group in the structure of GPPE. The carbonyl group is close to the oxyanion hole and can form the hydrogen bond with nitrogen atom of Gly193. The catalytic triad is destroyed and the hydroxyl group of Ser195 is turned towards the oxyanion hole, which seems to be important for selectivity.

In conclusion it can be said that although the GPPE conformation in the picrate structure differs from that in the uPA complex, the hydrogen bonds formed by GPPE in both structures are quite similar. Thus, the character and directions of the inhibitor—protein hydrogen bonds may be predicted from those found in the inhibitor simple salt structure.

Acknowledgements

We thank Professor Dr. Robert Huber for making it possible to determine the structure of GPPE in complex with uPA in his laboratory in Max-Planck-Institute in Martinsried, Germany. The authors are grateful to Dr. Uwe Jacob for his help in diffractometric measurements of GPPE in complex with uPA.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.020.

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