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The effects of amino-acid mutations on specific interactions between urokinase-type plasminogen activator and its receptor: *Ab initio* molecular orbital calculations

Shingo Tsuji[a], Tomoyo Kasumi[a], Keisuke Nagase[a], Eri Yoshikawa[a], Hiroshi Kobayashi[b], Noriyuki Kurita[a,*]

[a] *Department of Computer Science and Engineering, Toyohashi University of Technology, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan*
[b] *Department of Obstetrics and Gynecology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8521, Japan*

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ABSTRACT

During cancer invasion, the binding of urokinase-type plasminogen activator (uPA) to its receptor (uPAR) on the surface of a cancer cell is considered a trigger for invasion. Here, we present a stable structure of the solvated complex formed between uPA and uPAR (uPA–uPAR) and investigate the specific interactions between uPA and uPAR by *ab initio* fragment molecular orbital (FMO) calculations. The result indicates that the electrostatic interactions between the charged amino acid residues existing in both uPA and uPAR make a large contribution to the binding between uPA and uPAR. In particular, Lys23, Lys46, Lys98 and Lys61 of uPA are found to have strong attractive interactions with uPAR. To elucidate the effect of these residues on the interactions between uPA and uPAR, we substituted each of them with the uncharged amino acid Leu and investigated the interactions between the mutated uPA and wild-type uPAR. The interaction energies indicate that Lys46 and Lys98, which bind uPA to the rim of the central ligand-binding cavity of uPAR, make greater contributions to the binding between uPA and uPAR than Lys23, which is positioned at the bottom of the ligand-binding cavity of uPAR. The effect of hydrating water molecules located between uPA and uPAR is also investigated to be significant for the specific interactions between uPA and uPAR. These results are expected to be informative for developing new peptide antagonists that block the binding of uPA to uPAR.

1. Introduction

The number of diagnosed deaths due to cancer has been steadily increasing. Cancer cells can spread within internal organs of the body by metastasis and quickly proliferate, leading to a decrease in the number of healthy cells. Finally, cancer patients lose vital body functions, leading to death. If cancer metastasis could be inhibited, it is expected that the recovery rate for cancer patients would rapidly improve. Thus, it is necessary to develop medicines that suppress cancer metastasis. These medicines are usually administered to cancer patients after removing cancerous tumors by surgery. After surgery, cancer patients have such diminished physical strength that they cannot tolerate medicines with strong side effects such as is typical of anti-cancer agents. Therefore, it is essential to develop medicines with relatively few side effects [1].

During the processes of invasion and metastasis, cancer cells produce various proteases to dissolve cell tissues such as extracellular matrix (ECM) and basement membrane and to facilitate invasion into blood vessels. The cancer cell passes through the blood vessel to reach the endothelial cells of other organs and invades these cells to develop a new metastatic focus [2]. By repeating this process, cancer cells grow proliferously. The inhibition of cancer invasion is expected to suppress cancer metastasis, resulting in good prognoses for the cancer patients.

Recent biochemical experiments have elucidated many types of proteases playing essential roles in the invasion mechanism of cancer cells [3]. Among these proteases, urokinase-type plasminogen activator (uPA) [4,5] has been found to play a key role in cancer invasion and metastasis. The specific binding of the amino-terminal fragment (ATF) of uPA to the uPA receptor (uPAR) existing on the surface of cancer cells is considered a trigger for promoting cancer invasions. The cancer cells assemble uPARs in direction they are moving and bind uPA to uPAR. The uPA that is bound to uPAR efficiently converts inactive plasminogen existing on the surface of cancer cells into the active serine protease plasmin, which directly or indirectly dissolves ECM components [6]. Therefore, blocking uPA–uPAR binding is expected to inhibit cancer invasions. Based on this idea, Kobayashi et al. synthesized the chimeric protein ATF-HI-

* Corresponding author. Tel.: +81 532 44 6875; fax: +81 532 44 6875.
E-mail address: kurita@cs.tut.ac.jp (N. Kurita).

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Table 1
Amino acid residues of uPAR missing in each PDB structure.

PDB ID	3BT1	3LAQ	2FD6	2I9B (monomer 1, 2)
Missing amino acid residues of uPAR	83–84	82–92 227–231	81–91 130–139 249–251	106–109 132–136 246–249

8 [7], which is composed of bikunin, found in amniotic fluid, and the ATF of uPA. The effectiveness of this chimeric protein on inhibiting cancer invasion has been confirmed by the biological studies using laboratory animals [1]. Recently, we [8,9] obtained stable structures of ATF-HI-8 in water and investigated their electronic properties using semiempirical molecular orbital (MO) calculations. Based on the computed results, we have proposed parts of ATF-HI-8 that are important for the specific interactions between ATF-HI-8 and uPAR.

In previous structural analyses [10,11] of the complex between uPA and uPAR, it was elucidated that the ATF of uPA, which is composed of amino acids 1–132 of uPA, binds specifically to uPAR. In particular, the Ω-loop (residues 19–31) of ATF was found to be responsible for the high-affinity interactions between uPA and uPAR [12]. In our previous molecular simulations [13], the specific interactions between uPA and uPAR were investigated using *ab initio* MO calculations based on the fragment MO (FMO) method [14–17].

In the present study, we have built upon our previous study [13] to obtain stable calculated structures of the solvated uPA–uPAR complex using different experimental structures to confirm the adequacy of the present molecular simulations. From the obtained interaction energies, the amino acid residues of uPA that are important for the specific interactions between uPA and uPAR have been elucidated. Furthermore, some of these important residues of uPA are mutated, and the change in the specific interactions between uPA and uPAR induced by the mutations are investigated using the *ab initio* FMO calculations. From the computed results, we attempt to propose peptides that can bind specifically to the uPA-binding site of uPAR and efficiently inhibit cancer invasion and metastasis.

2. Details of molecular simulations

2.1. Construction and optimization of a solvated structure of the uPA–uPAR complex

Four structures of the uPA–uPAR complex are registered in the Protein Data Bank (PDB). In these structures, the positions of some amino acid residues cannot be observed. Table 1 lists the missing amino acid residues in each PDB structure. In our previous simulations [13], the X-ray crystal structure with PDB ID 2I9B was employed. For our current calculations, we have employed the newest X-ray structure with PDB ID 3BT1 [18] because this structure is only missing two amino acids 83 and 84 in uPAR. This structure is of uPAR (residues 1–275) in complex with the ATF (residues 8–132) of uPA, vitronectin and other co-factors. The experiment [18] indicates that there is no direct contact between uPA and vitronectin and that the structure of the uPA–uPAR interface is not perturbed by the binding of vitronectin. Thus, we extracted the uPA–uPAR structure from this PDB structure. In addition, by using the homology modeling program SWISS-MODEL [19], we predicted the structures of these missing residues and added them into the PDB file to obtain an initial structure for the complete uPA–uPAR complex.

The N- and C-termini of uPA and uPAR were terminated by NH_3^+ and COO^-, respectively. To accurately replicate the state of the uPA–uPAR complex existing on the surface of a cancer cell, we added solvating water molecules in an 8 Å shell around the complex and considered them explicitly in our calculations. This solvated structure was optimized using the classical molecular mechanics (MM) program AMBER9 [20], in which the Parm99 [21] and TIP3 [22] force fields were used for the uPA–uPAR structure and the water molecules, respectively. The threshold value of the energy-gradient for the convergence of the AMBER9 optimization was set to 0.001 kcal/mol/Å.

2.2. Ab initio FMO calculations for the solvated uPA–uPAR complex

The solvated structure of uPA–uPAR has approximately 2600 water molecules, so it is not practical to calculate its electronic properties using the *ab initio* MO method. We thus considered only the solvating water molecules existing within 5 Å from amino acid residues 17–41 of uPA and amino acid residues 24–70, 122–171 of uPAR. In the previous experiment [12], these amino acid residues were found to contribute to the specific interactions between uPA and uPAR. This solvated uPA–uPAR complex has 436 water molecules.

To investigate the binding energy between uPA and uPAR, the solvated uPA–uPAR structure was divided into the following four structures.

1. uPA–uPAR complex containing solvating water molecules (complex).
2. uPA containing solvating water molecules (uPA + water).
3. uPAR containing solvating water molecules (uPAR + water).
4. Solvating water molecules (water).

From the total energies (T.E.) obtained from the FMO calculations, the binding energy (B.E.) between uPA and uPAR mediated by water molecules was estimated as

$$\text{B.E.} = -\text{T.E.(Complex)} + \text{T.E.(uPA + water)} + \text{T.E.(uPAR + water)} - \text{T.E.(water)}.$$

It must be noted that the basis set superposition error was neglected in the analysis of the binding energy between uPA and uPAR.

For the FMO [14–17] calculations, we used the ABINIT-MP program [23] developed by Nakano et al. The MP2 [24,25] method and the 6-31G basis-set were employed in the FMO calculations. One of the advantages of FMO is that FMO can obtain the interaction energies between the fragments using one SCF calculation while considering the effect of the surrounding fragments. This pair interaction energy obtained by FMO is somewhat similar to the simple pair interaction energy computed by classical force field methods. However, in the FMO evaluation of the pair interaction energy, the influence of the other fragments is taken into account as a direct coulomb interaction. In the present study, the fragment size was set as one amino acid residue or one water molecule. We thus investigated the interaction energies between each amino acid residue of uPA and uPAR, to elucidate which amino acid residues are important for the binding between uPA and uPAR. The effect of solvating water molecules on the specific interactions between uPA and uPAR was also investigated.

In addition, to analyze the effect of uPA amino acid residues on the specific interactions between uPA and uPAR, some of the important residues in uPA were mutated to other uncharged amino acids, and the solvated structure of the mutated uPA–uPAR complex was optimized using the classical MM method in AMBER9. For the optimized structure, the specific interactions between the mutated uPA and uPAR were investigated using the FMO method to determine the effect of the mutations on the interactions. From these computed results, we clarified which amino acid residues of uPA are