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Novel binding patterns between ganoderic acids and neuraminidase: Insights from docking, molecular dynamics and MM/PBSA studies

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ABSTRACT

Recently, ganoderic acids (GAs) give rise to the attractive candidates of novel neuraminidase (NA) inhibitors. However, there is still no evident conclusion about their binding patterns. To this end, docking, molecular dynamics and MM/PBSA methods were combined to study the binding profiles of GAs with the N1 protein and familiar H274Y and N294S mutations (A/Vietnam/1203/04 stain). It was found that the binding affinities of ganoderic acid DM and Z (ΔG_{bind}, −16.83 and −10.99 kcal mol^{-1}) are comparable to that of current commercial drug oseltamivir (−23.62 kcal mol^{-1}). Electrostatic interaction is the main driving force, and should be one important factor to evaluate the binding quality and rational design of NA inhibitors. The 150-loop residues Asp151 and Arg152 played an important role in the binding processes. Further analysis revealed that ganoderic acid DM is a potential source of anti-influenza ingredient, with novel binding pattern and advantage over oseltamivir. It had steric hindrance on the 150 cavity of N1 protein, and exerted activities across the H274Y and N294S mutations. This work also pointed out how to effectively design dual-site NA inhibitors and reinforce their affinities. These findings should prove valuable for the in-depth understanding of interactions between NA and GAs, and warrant the experimental aspects to design novel anti-influenza drugs.

1. Introduction

Influenza is an infectious disease caused by influenza virus, which is negatively stranded RNA virus of the orthomyxoviridae family [1]. Some types of influenza virus can cause acute respiratory infections to humans, and may result in annual epidemics and infrequent pandemics [2,3]. Highly pathogenic influenza A/H5N1 virus is one of the most dangerous ones, with quickly expanding host reservoir and significant pathogenicity in humans [4]. To date, commercial neuraminidase (NA) inhibitors are first-line drugs and probably the most effective strategy to combat influenza infection [5]. However, the emergence of drug-resistant mutants (virus with amino acid substitutions in NA, *e.g.*, H274Y and N294S mutants) has aroused high concerns in the development of medications [6,7].

Neuramindiase (NA) is a surface glycoprotein of influenza virus, which enzymatically cleaves the terminal neuraminic acid residues from host glycosylated receptors [8]. During virus replication, it is of great relevance for the release of progeny virions from infected cells and preventing their self-aggregation [9]. Owing to the essential roles, blocking the NA function with inhibitors has ranked as an attractive way for structure-based drug designs (SBDD) that have resulted in the production of commercial drugs zanamivir (Relenza) and oseltamivir (Tamiflu) [5,10]. It is well known that, in NA-inhibitor complexes, the Arg clusters (118, 292 and 371) of NA binding pocket generally form salt bridges with anionic substituents of inhibitors (Fig. 1, in N2 numbering), such as carboxylate anion of oseltamivir carboxylate (OC) [11–13]. 150-loop residues Asp151 and Arg152 provide a hydrogen-binding environment, and residue Glu276 joins with Glu277 to create a mixed polarity pocket [10]. On the other hand, framework residues Glu119, Asp198, Ile222, His274, Asn294 and Glu425 show stabilization effects to the docked complexes by impacting on the above binding-pocket residues. Mutations of these residues might significantly affect the binding properties, and then confer drug resistances, for example, H274Y and N294S could confer oseltamivir resistances in H5N1 viruses [7,14].

Ganoderma lucidum has been used as a Chinese traditional folk for centuries [15]. Among its constituents, ganoderic acids (GAs)

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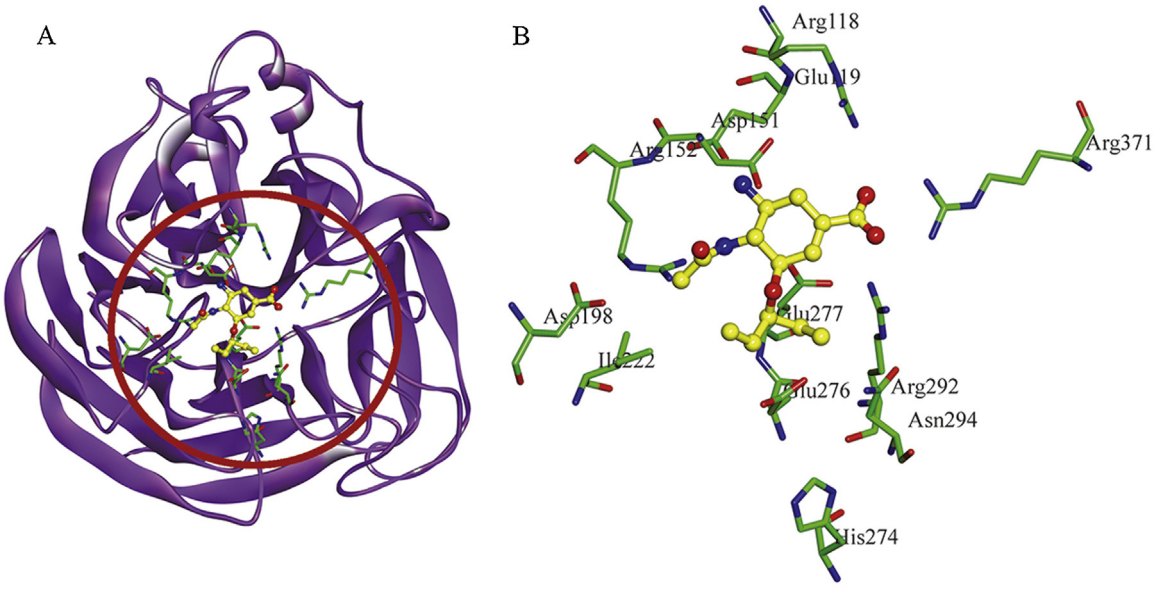


Fig. 1. A) Ribbon schematic representation of wild-type N1 ($N1^{WT}$) in complex with oseltamivir carboxylate (PDB entry 2HU0). B) Close up view of the binding-pocket residues. Oseltamivir carboxylate (OC) is represented by ball and stick model. Catalytic residues Arg118, Asp151, Arg152, Glu276, Arg292 and Arg371 have direct interactions to OC; framework residues Glu119, Asp198, Ile222, His274, Glu277, Asn294 and Glu425 show stabilization effects to the docked complex. The C, N, O atoms are colored in yellow, blue and red for OC whereas in green, blue and red for the residues, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

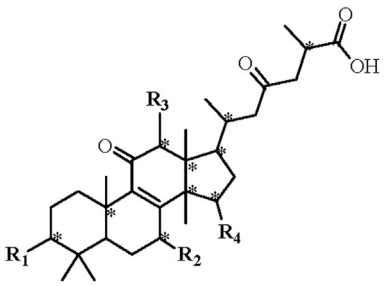
are responsible for a number of physiological effects including immunomodulation, antitumor and antibiosis [16,17]. To date, there is considerable information on using GAs as an alternative adjuvant in the treatment of influenza [17,18]. While we were preparing the manuscript, *in vitro* NA inhibition assay revealed that GAs have impressive inhibition activities (at 200 μM) against different NA subtypes, even the oseltamivir-resistant cases (N294S) [17]. While, most of them have more adverse effect on NA subtype 1 (N1), for example, the inhibition rates of ganoderic acid DM (DM hereafter, structure with one double bond ($\Delta^{8,9}$) and a branch with carboxylate anion, (Scheme 1) against N1 proteins are more than double the rates of other NAs [17].

Albeit, virologic experiments have foreshadowed the inhibitory effects of GAs against various NA proteins [17,18], however, their interactions still require systematical exploration. Currently, docking, molecular dynamics (MD), and molecular mechanics–Poisson–Boltzmann/surface area (MM/PBSA) methods have been extensively used to expound the interactions between inhibitors and the NA binding pockets [5,19] and identify the novel druggable 150-loop domain in NA [20]. Therefore, these approaches were utilized to study the interaction profiles of selected GAs (Scheme 1) with wild-type A/H5N1 NA and two mutants (H274Y and N294S). We anticipate that the results will provide clues on interactions between GAs and NA proteins, as well as the development of NA inhibitors with minimized viral resistance.

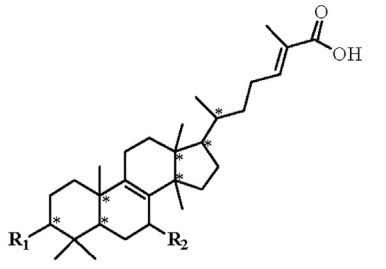
2. Materials and methods

2.1. System preparations

In accord to our previous reports [21,22], the oseltamivir-soaked structures of A/H5N1 WT and mutants H274Y and N294S (derived from A/Vietnam/1203/04 stain) were obtained from the RCSB Protein Data Bank (http://www.rcsb.org), with entry codes 2HU0 (Chain B) [23], 3CL0 [6] and 3CL2 (Chain B) [6], respectively. For convenience, these NA monomers are named as $N1^{WT}$, $N1^{H274Y}$ and $N1^{N294S}$ throughout this work. The calcium ion (Ca^{2+}) near the binding pocket was kept [21]. The hydrogen atoms were added to amino



Compound	R1	R2	R3	R4
A	= O	-OH	-H	-OH
B	-OH	-OH	-H	= O
C1	= O	-OH	-H	= O
D	= O	-OH	-H	= O
F	= O	= O	$-OCOCH_3$	= O
G	-OH	-OH	-OH	= O
H	-OH	= O	$-OCOCH_3$	= O



Compound	R1	R2
DM	= O	= O
Z	-OH	-H

Scheme 1. Chemical structures of ganoderic acids used in this study.