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STUDY OF THE AFRICAN SWINE FEVER VIRUS DNA POLYMERASE X 5'-P INTERACTION SITE POSSIBLE INHIBITORS

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African swine fever (ASF) virus is one of the most devastating diseases of domestic pigs for which no effective vaccines are available. Flavonoids, natural products isolated from plants, have been reported to have significant *in silico*, *in vitro* and *in vivo* antiviral activity against different viruses.

ASF – ASFV – PolX – flavonoids – in silico

Խոզերի աֆրիկյան ժանտախտի վիրուսը (ԽԱԺՎ) ընտանի խոզերի ամենակործանարար հիվանդություններից է, որի դեմ արդյունավետ պատվաստանյութեր դեռ գոյություն չունեն: Ցույց է տրված ֆլավոնոիդները՝ բույսերից զատված բնական նյութերը, ցուցաբերել են զգալի հակավիրուսային ակտիվություն տարբեր վիրուսների դեմ *in silico*, *in vitro* և *in vivo* փորձերի ժամանակ:

ԽԱԺ – ԽԱԺՎ – Pol X – ֆլավոնոիդներ – in silico

Вирус африканской чумы свиней (ВАЧС) является высококонтагиозным вирусным заболеванием, против него не имеется ни эффективного лечения, ни вакцин. Показано, что натуральные продукты выделенные из растений флавоноиды, обладают значительной противовирусной in silico, in vitro и in vivo активностью против различных вирусов.

АЧС – ВАЧС – Pol X – флавоноиды – in silico

African swine fever (ASF, Montgomery disease) is an acute infectious viral disease of domestic and wild pigs, caused by DNA-containing virus of the Asfarviridae family, genus Asfavirus. The disease develops in asymptomatic, super-acute, acute, subacute and, less commonly, chronic forms. Domestic and wild pigs are susceptible to the disease, regardless of age, breed and season [1-3].

According to a number of authors in the Caucasus, the disease was registered in Georgia in March – April 2007 [4].

The first outbreak on the territory of Armenia was registered on August 6, 2007 in the northern region of the republic, bordering with Georgia. The agent of disease most likely penetrated from Georgia. ASF could be brought in by lawful transportation or smuggling of pigs and pork products, from moving freely across the border domestic pigs or wild boars. Most outbreaks were recorded in the same northern regions, bordering Georgia. The last officially confirmed outbreak occurred in May 2008.

Ongoing surveillance focuses on four previously infected areas woodlands where pigs graze.

On August 16, 2018, the National Center for Animal Health and Epidemiology of China recorded an outbreak of African swine fever in Henan Province, an area in the east part of China.

ASF is an economic disaster for the pig industry due to the following reasons:

- high contagiousness and mortality (mortality of this disease can reach 100% while surviving animals remain lifelong virus carriers);
- lack of vaccine. As a result, the only way to eliminate it is the mass slaughter of susceptible animals in the threatened zone, a total ban on any movement of products made of pork, in addition to the huge costs of containing and eliminating the infection, which, in general, is a disaster for the cattle raising industry [5-7].

DNA polymerase X in African swine fever virus.

African swine fever virus contains the smallest DNA polymerase X (PolX). PolX, belonging to the X family, is an important participant in excision repair of bases. PolX is able to correctly restore the DNA structure after removal of the "wrong" bases using DNA glycosylases, and carries out DNA synthesis in areas containing "gaps".

PolX is a slow-acting enzyme that lacks 3'-5'-exonuclease activity, it also has a very low pyrophosphorolysis activity. It is another feature is the presence of additional activity of removing deoxyribosylphosphate residue during DNA repair [8].

PolX consists of 174 amino acid residues and has a molecular weight of 20 KDA. It is about half the size of the smallest known DNA Polymerase β (Pol β).

The experimentally studied tertiary structures of DNA PolX obtained by NMR [9] and X-ray diffraction analysis [9] showed high similarity to the Palm and Thumb domains of Pol β. Figure 1 shows the tertiary structure of Pol X: the N-terminal Palm domain consisting of 10 β-sheets (1-10) and 3 α-helices (A-C); C-terminal Thumb domain consisting of 3 β-sheets (11-13) and 3 α-helices (D-F).

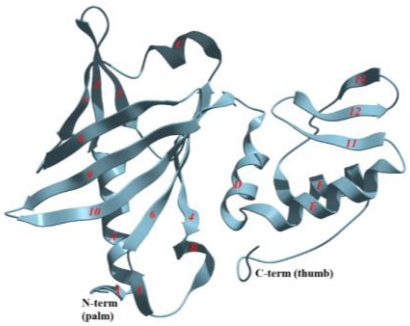


Fig. 1. Tertiary structure of DNA Polymerase X

In 2017 it was found, that the PolX substrate has a phosphate group (5'-P) at the 5′ end with which PolX binds to DNA. Previous kinetic studies have shown that 5'-P can significantly increase the catalytic activity of PolX. In homologous structures, the 5'-P groups interacted with the 8-KD domain, which is not happening in PolX [9].

A 5'-P binding site was found in PolX, which is located in the Thumb domain. The 5'-P binding site is positively high charged. Two Arg residues (Arg125 and Arg168)

and one Thr residue (Thr166) are involved in pocket formation and they form five hydrogen bonds with 5'-P.

The amino acids Arg125 and Arg168 are variable in the PolX family. Although homologous proteins have Arg residues, for example, TtPolX has Arg268 (corresponding to Arg125 AsfvPolX), and hsPolβ and RatPolβ have Arg328 (corresponding to Arg168 of PolX), none of them have two Arg amino acids in corresponding positions, which indicates 5'-P is a must-have and unique pocket for PolX. [10]

A superposition of the tertiary structures of all the studied DNA polymerases showed, that the 5'-P pocket can undergo large conformational changes in the absence of 5'-P DNA. Therefore, we can conclude, that the inhibition of 5'-P may result in obstruction of the binding of viral DNA and subsequent repair of damaged areas.

All the abovementioned suggests that ASFV-Pol-X can be a good candidate for in silico antiviral screening.

Flavonoids

Flavonoids are polyphenolic compounds of plant origin. At present, the spectrum of action of these compounds in the human body is determined, they are anti-inflammatory, antifungal, antibacterial, antiviral, anticarcinogenic, nephroprotective, hepatoprotective. The established properties of flavonoids open up wide possibilities for their use as medicines, which do not have serious side effects, unlike synthetic analogues [11].

AIM AND OBJECTIVES

The research was aimed at studying the effects of phenolic compounds on the PolX protein tertiary structure for clarifying their antiviral activity against ASFV**.**

To achieve the goal, the following objectives were set:

1. Carry out the search of all available flavonoids and their analogues structures to create a compounds library.
2. Carry out a virtual ligand screening and molecular docking simulation of compounds library with PolX in 5'-P binding site.

Materials and methods. The determination of PolX ligand-binding pockets, a search of flavonoids and their derivatives structures, virtual ligand screening (VLS) and visualization were performed, using the software package ICM-PRO 3.8-5 [12, 13]. In conducted experiments, the structure of PolX was used, obtained by X-Ray [PDB ID: 5HRI], which was retrieved from Protein Data Bank.

Based on the basic chemical structures of all classes and subclasses of flavonoids, a substructure search was performed in the MolCart database, on the basis of which has been created a library of flavonoids and their derivatives, containing 26618 compounds. Also, all Metabolomics (6850 compounds) and Phenol-Exploere [14] (523 compounds) databases have been used. These libraries have been merged and duplicates have been removed from them. The final library of flavonoids and their derivatives contains 33325 compounds.

Results and Discussion.

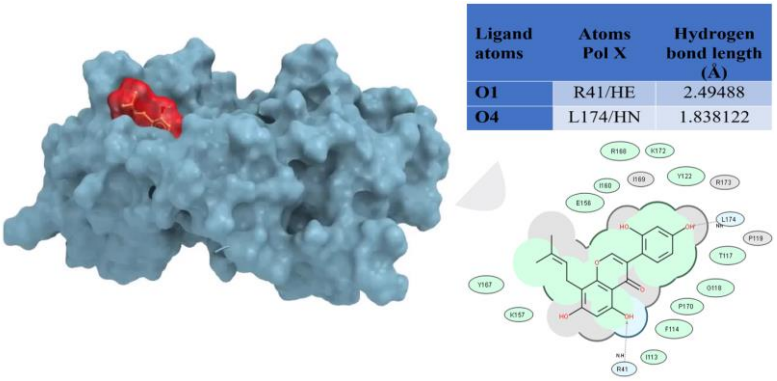
Virtual ligand screening of flavonoids and their derivatives.

The structure of PolX protein for in silico experiments, obtained by retrieving the three-dimensional crystal structure from the Protein Data Bank (PDB ID: 5HRI) and the studied interaction pocket, have been used for docking and VLS with the obtained library of flavonoids and their derivatives. VLS has been conducted in the condition of full ligand flexibility. From 33325 compounds, 3 showed good results with interaction energy less than -32 ICM Score, which is considered as minimum allowable interaction

energy in the ICM-PRO program. For these compounds, the VLS and docking experiments have been independently repeated 10 times.

Inhibition of PolX 5'-P interaction site.

2,3-Dehydrokievitone interacts with -50.68 ICM Score, forming 2 hydrogen bonds in the 5'-P interaction pocket of PolX (fig.2).

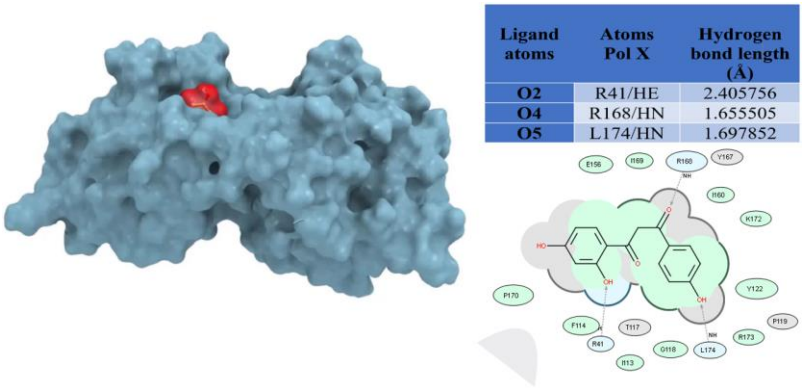


Ligand atoms	Atoms Pol X	Hydrogen bond length (Å)
O1	R41/HE	2.49488
O4	L174/HN	1.838122

Fig. 2. Localization and interaction sites of 2,3-Dehydrokievitone in Pol X 5'-P pocket.

From interaction sites of 2,3-Dehydrokievitone with PolX should be highlighted Leu174, which is necessary for the interaction of PolX with DNA. The localization of 2,3-Dehydrokievitone in the 5'-P interaction pocket can lead to inhibition of the PolX-DNA complexation.

Licodione interacts with -46.88 ICM Score, forming 3 hydrogen bonds in the 5'-P interaction pocket of PolX (fig.3).



Ligand atoms	Atoms Pol X	Hydrogen bond length (Å)
O2	R41/HE	2.405756
O4	R168/HN	1.655505
O5	L174/HN	1.697852

Fig. 3. Localization and interaction sites of Licodione in Pol X 5'-P pocket.

Arg168 and Leu174 should be highlighted from interaction sites of Licodione with PolX, which is necessary for the interaction of PolX with DNA. The localization of Licodione in the 5'-P interaction pocket can lead to inhibition of the PolX-DNA complexation.

Myricetin interacts with -46.79 ICM Score, forming 3 hydrogen bonds in the 5'-P interaction pocket of PolX (fig.4).

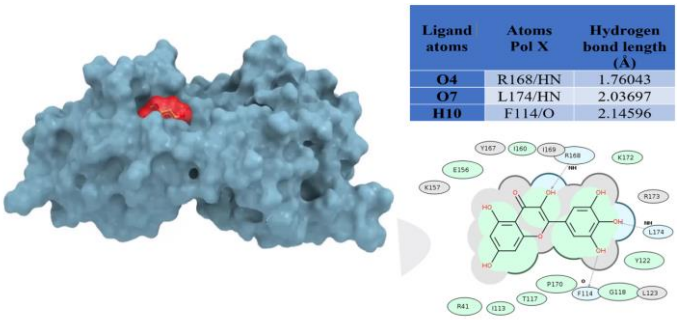


Fig.4. Localization and interaction sites of Myricetin in Pol X 5'-P pocket.

Phe114, Arg168 and Leu174 should be highlighted from interaction sites of Myricetin with PolX, which is necessary for the interaction of PolX with DNA. The localization of Myricetin in the 5'-P interaction pocket can lead to inhibition of the dGTP-DNA complexation. Myricetin, like the above compounds, theoretically can lead to inhibition of the 5'-P pocket, which can lead to inhibition of the PolX-DNA interaction and to disruption of the viral DNA reparation process.

As a result of the *in silico* experiments, in particular, virtual ligand screening and molecular docking, polyphenolic compounds have been identified, they have plant origin with an inhibitory effect on the tertiary structure of PolX.

The following conclusions may be drawn from the results of our study:

1. The flavonoids have been identified, which showed a high degree of interaction with the 5'-P pocket of PolX, which is necessary for the interaction of PolX with DNA.
2. The obtained results showed the possibility of using polyphenolic compounds as inhibitors of PolX and as possible therapeutic agents against ASFV.

Acknowledgements

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