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Spectral and theoretical study of SARS-CoV-2 ORF10 protein interaction with endogenous and exogenous macroheterocyclic compounds

M.O. Koifman a,b, A.S. Malyasova a, Yu.V. Romanenko a, E.S. Yurina b, N.Sh. Lebedeva b, Yu.A. Gubarev b,* and O.I. Koifman a,b

a Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russia
b G.A. Krestov Institute of Solution Chemistry of the Russian Academy of Sciences, 153045 Ivanovo, Russia

HIGHLIGHTS

- Molecular docking study of 3 macrocyclic compounds with QRF10 of SARS-CoV-2 was shown.
- Protoporphyrin and Fe(III) protoporphyrin form several types of complexes with the ORF10 protein.
- The results of theoretical studies were confirmed by spectral data (UV–Vis, IR spectroscopy).
- Chlorin forms with ORF10 more stable complexes than protoporphyrins.

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GRAPHICAL ABSTRACT

ABSTRACT

The coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 coronavirus has spread rapidly around the world in a matter of weeks. Most of the current recommendations developed for the use of antivirals in COVID-19 were developed during the initial waves of the pandemic, when resources were limited and administrative or pragmatic criteria took precedence. The choice of drugs for the treatment of COVID-19 was carried out from drugs approved for medical use. COVID-19 is a serious public health problem and the search for drugs that can relieve the disease in infected patients at various stages is still necessary. Therefore, the search for effective drugs with inhibitory and/or virucidal activity is a paramount task. Accessory proteins of the virus play a significant role in the pathogenesis of the disease, as they modulate the host's immune response. This paper studied the interaction of one of the SARS-CoV-2 accessory proteins ORF10 with macroheterocyclic compounds – protoporphyrin IX d.m.e., Fe(III) protoporphyrin d.m.e. and 5,10,15,20-tetrakis(3'-pyridyl)chlorin tetraiodide, which are potential inhibitors and virucidal agents. The SARS-CoV-2 ORF10 protein shows the highest affinity for Chlorin, which binds hydrophobically to the alpha structured region of the protein. Protoporphyrin is able to form several complexes with ORF10 close in energy, with alpha- and beta-molecular recognition features; while Fe(III) protoporphyrin forms complexes with the orientation of the porphyrin macrocycle parallel to the ORF10 alpha-helix. Taking into account the nature of the interaction with ORF10, it has been suggested that Chlorin may have virucidal activity upon photoexposure. The SARS-CoV-2 ORF10 protein was expressed in Escherichia coli cells, macroheterocyclic compounds were synthesized, and the structure was confirmed. The interaction between macrocycles with ORF10 was studied by spectral methods. The results of in silico studies were confirmed by experimental data.

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1. Introduction

COVID-19, caused by SARS-CoV-2, spreads very quickly and has a high pathogenicity. Recently, the search for new drugs against coronavirus has been actively conducted [1–3] and its structure is being studied. Numerous studies have shown that the SARS-CoV-2 virus contains single-stranded RNA and 28 specific proteins grouped into 16 non-structural proteins (Nsp1 to Nsp16), four structural proteins (E, M, N, and S) and eight accessory proteins (ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c and ORF10) [4–7]. It is known that accessory proteins are not critical for viral replication, but determine strategies for evading the virus from the host’s immune response. It is believed that the main mechanism of their action is the modulation of protein–protein interactions of the host [8]. Therefore, it is necessary to constantly update knowledge about the functions and structure of accessory proteins, which will allow us to propose compounds that can inactivate the work of accessory SARS-CoV-2 proteins and thereby influence the pathogenesis of the disease. Consider the available information about ORF10. ORF10 is the smallest accessory protein in SARS-CoV-2, consisting of 38 amino acid residues. The crystallographic structure of the ORF10 protein has not yet been determined. Studies attempting to recognize the secondary structural elements of this protein indicate the presence of one α-helix (11–21), two β-sheets (4–8 and 26–34) and several disordered fragments in the protein [9]. Structurally, the ORF10 model is represented by the β-α-β motif spanning residues 3–31. Electrostatic surface map showing two areas of positive charge and one area of negative charge [9]. The ORF10 protein has two hydrophobic regions, including amino acid residues 3–19 and 28–36, and one hydrophilic region (residues 20–27) [9].

An analysis of the evolution of the genome segment encoding ORF10 in coronaviruses [10] showed that the ORF10 protein is specific for SARS-CoV-2, since it is not present in SARS-CoV and is a functional protein in SARS-CoV-2 [11]. This indicates positive selection in its evolution and opens up prospects for the unmistakable identification of the SARS-CoV-2 virus by the ORF10 protein. However, the segment of the ORF10 genome is mutable [12], and most of the identified mutations are aimed at reducing the stability of the structure of the encoded protein, which should allow the ORF10 protein to more effectively interact with other proteins [10,13].

ORF10 contains a Molecular Recognition Features (MoRF) region in the first β-sheet, comprising amino acid residues 3 to 7 at the N-terminus [14]. According to [14], this is the site that allows the ORF10 protein to adopt a set of conformations for binding to various host proteins [15]. A similar conclusion was reached in [9], which reported that the amino acid residues in the N-terminal half of ORF10 (1–14) are more prone to interact with proteins than the amino acid sequence of the C-terminus.

Indeed, the presence of unstructured regions, two short beta chains and one hydrophobic alpha chain provides good conditions for ORF10 binding to a number of proteins. For example, it was shown [8] that SARS-CoV-2 ORF10 interacts with components of the cullin-2 (CUL2) RINGE3 ligase complex, in particular, with the CUL2zag11b complex. This interaction is mediated by amino acid residues (β-MoRF) at the N-terminus of the protein [9]. ORF10 binds to the CUL2zag11b complex for ubiquitination and degradation. It should be noted that the capture of ubiquitination pathways is a common strategy of viruses necessary for their replication and pathogenesis [16]. On the other hand, the ZYG11B complex can bind to the N-terminal glycine in ORF10 to target it for degradation – this is the antiviral strategy of the host organism [17].

The work [13] reported that the partially disordered structure of SARS-CoV-2 ORF10 allows it to bind to the major histocompatibility complex I (MHC-I), modulating the T-cell response. This thesis is confirmed by the subcellular localization of ORF10, which can be located in the cytoplasm, but most of it is localized in the endoplasmic reticulum [18], which is necessary for interaction with MHC-I [13].

The ORF10 protein contains a large number of random epitopes of cytotoxic T-lymphocytes, primarily on the α-helix [13,19]. Therefore, high levels of ORF10 expression can lead to an increased immune response and provoke a cytokine storm. This circumstance was used to consider a vaccine based on a multi-epitope, including ORF10 epitopes [20,21]. Thus, it has been practically proven that SARS-CoV-2 ORF10 affects the pathogenicity of the virus [13,22–24].

The α-helix of the ORF10 protein has been identified as a possible transmembrane (TM) helix spanning residues 3–19, notably, it also constitutes the hydrophobic region of the ORF10 protein noted above [9,10]. Whether ORF10 is integrated within the membrane or peripheral to it remains unclear, but given the number of nonpolar residues, the authors of [25] suggest that ORF10 is integrated within the membrane. The SARS-CoV-2 genome encodes several viroporins. For example, the ORF3a protein is a viroporin and oligomerizes to form ion channels inside mitochondrial membranes that cause cell death [10,18,26]. The authors of [25] suggest that SARS-CoV-2 ORF10 can also oligomerize in such a way that polar and electrically charged residues cover the inside of the pore, which facilitates the transport of ions or small molecules. As noted above, most ORF10 is concentrated in the endoplasmic reticulum (ER) [18], in which case the ORF10 protein can bind to ER membranes.

Considering the possible mechanisms of action of ORF10, we should mention the proposed theory [27,28] about the direct interaction of ORF10 and ORF3a proteins with hemoglobin, which, according to the authors, results in heme demetalization and binding of protoporphyrin IX. ORF10 plays a major role in hemoglobin unfolding. It should be noted that the article [28] is in the ChemRxiv preprint state, and the question of the direct interaction of accessory SARS-CoV-2 proteins with hemoglobin has not been unambiguously proven, but not refuted, and taking into account the structural features of the ORF10 protein, it is quite probable. In [29], the interaction of SARS-CoV-2 proteins with heme was proved.

In general, the available data suggest that the ORF10 protein may be multifunctional. However, experimental information is clearly not enough to describe and characterize the properties of ORF10. Therefore, the aim of this work was a theoretical and experimental study of the ORF10 protein and its complexes with macroheterocyclic compounds in order to search for a potential ORF10 inhibitor and/or virucidal agents. At this stage of the work, endogenous porphyrins – protoporphyrin IX d.m.e., iron(III)chloride protoporphyrin IX d.m.e., and exogenous 5,10,15,20-tetrakis(3-pyridyl)chlorin tetraiodide – were evaluated as inhibitors. The first two compounds are of interest as biologically active substances that can affect the life cycle of the virus, and Chlorin is an approved drug for photodynamic therapy and, if effective, can be repurposed for the treatment of COVID19.

2. Materials and methods

2.1. Reagents and materials

ORF10 was expressed based on pGBW-m406950, which was a gift from Ginkgo Bioworks & Benjie Chen (Addgene plasmid...
149258; http://n2t.net/addgene:149258; RRID: Addgene_149258). The procedure is described in detail in supplementary materials.

Phosphate-buffered saline (PBS) was used for solution preparing. Double distilled water was used for solution preparing. Freshly distilled DMF (Reachim, Russia) was used. KBr for IR spectroscopy (Sigma-Aldrich, Germany) was dried before using. Distilled DMF (Reachim, Russia) was used. KBr for IR spectroscopy. Double distilled water was used for solution preparing. Freshly distilled DMF (Reachim, Russia) was used. KBr for IR spectroscopy (Sigma-Aldrich, Germany) with

\[
\text{Fcorr} = \frac{F_{\text{corr}}}{C_0} - C_1
\]

was made in terms of the equation: \(\text{Fcorr}\) - corrected and observed fluorescence at the corresponding wavelength, \(A_{\text{em}(\lambda)}\) is the optical density of the solution at the excitation wavelength \(\lambda_{\text{ex}}\) and \(A_{\text{em}(\lambda)}\) is the optical density of the solution at the corresponding emission wavelength [31].

IR spectra were recorded on an Avatar 360 IR-Fourier spectrometer (Thermo Nicolet, USA) over the range of 4000–500 cm\(^{-1}\) by means of KBr pallets.

2.4. Molecular docking

Structure of ORF10 predicted by D-I-Tasser (QHI42199.pdb) was downloaded from Zhang Lab website [32,33]. The structures of the macroheterocyclic compounds (Fig. 1) were minimized in the ORCA 4.0 program [34] using the DFT method. Molecular docking of proteins with porphyrins was performed using AutoDock Vina 1.1.2 [35] and visualized with PyMol 2.4.1. The ligand and protein structure files were prepared using AutoDockTools 1.5.6. When preparing the structure of the ligand, rotating bonds were selected automatically. Polar hydrogens were added to the protein structure. The grid matrix was sized so that the protein molecule was completely overlapped. Due to the large size of the grid matrix, the exhaustiveness parameter was increased to 512 [36]. Molecular docking made it possible to find the 20 most favorable structures for each porphyrin. After analyzing the results, the most optimal positions indicated in Table 1 were selected. In the case of cationic and anionic macrocycles, the potential of the protein globule was accessorially calculated by the ABPS method to analyze the docking sites [37].

3. Results and discussion

3.1. Theoretical study. Molecular docking

The results obtained using the molecular docking method are presented in Table 1. Of greatest interest are the complexes of macroheterocyclic compounds (MHCs) with β-MoRF ORF10, i.e. with the participation of the N-terminus of the protein and the first β-sheet, as well as MHCs that bind to the α-helix [9,10].

Among the studied MHCs, only protoporphyrin (complexes 2 and 3) binds to β-MoRF ORF10. The binding affinity of protoporphyrin protein is relatively low (Table 1). Based on the obtained values, inhibition of the β-MoRF ORF10 region by protoporphyrin is unlikely. However, it should be noted that complex 2 does not form bonds between atoms of the aromatic macrocycle and the protein; therefore, PP in the complex with SARS-CoV-2 ORF10 can exhibit photocatalytic activity. The nitrogen atoms of the porphyrin macroring form H-bonds with amino acid residues of the protein in complex 4, the ability of PP to photocatalytically oxidize proteins will be reduced, since the absorbed light energy will also dissipate due to numerous vibrational components.

![Scheme 1. Structural formulas of MHCs.](image-url)
As shown above, the α-helix is the hydrophobic region of ORF10. Therefore, it is not surprising that all studied MHCs form complexes with this region of the protein (table). The binding affinity of MHCs increases in the series: PP (complex 1) < ClFePP (complex 1) < Chlorin. Chlorine, unlike the studied porphyrins, does not form H-bonds with the ORF10 protein; along with the highest binding energy, it is also suitable for photocatalytic protein oxidation. It should be noted that, according to calculations, the isoelectric point of ORF10 is 8.3 [38]. Therefore, at physiological pH values, the ORF10 protein will have a total positive charge, and at pH greater than 8.3, it will have a negative charge. The cationic nature of Chlorin under physiological conditions will allow SARS-CoV-2 ORF10 to be targeted.

### 3.2. Experimental study

Fig. 2 shows the UV–Vis spectrum of ORF10 in phosphate buffer at pH = 8. The extinction coefficient of the protein at the maximum (λ = 229 nm) was 85500 M⁻¹·cm⁻¹. The spectrum of the protein is asymmetric, the maximum is shifted towards the maximum of the absorption band of peptide bonds (190–220 nm). The absorption maximum of aromatic amino acids (275–282 nm) is not so clearly observed, which is logical, since ORF10 contains 7 aromatic amino acids, of which 3 are tyrosine and 4 phenylalanine residues, which have absorption maxima at wavelengths of 272 and 260 nm, respectively.

The fluorescence of proteins is usually excited by light with a wavelength of 280 nm for tyrosine residues and 295 nm for tryptophan residues. The low quantum yield of tyrosine fluorescence makes it difficult to detect the fluorescence of this residue. There are no tryptophan residues in the ORF10 sequence; therefore, the interaction of Chlorin with ORF10 was studied on the basis of UV–Vis spectra and Chlorin fluorescence.

![Fig. 1. ORF10 complexes with ClFePP, Chlorin and PP according to molecular docking data.](image)

![Fig. 2. UV–Vis spectrum of ORF10 (7.5·10⁻⁶ M) in PBS pH 8.0.](image)

### Table 1

Results of molecular docking of ORF10 with MHCs.

| MHCs  | N | Binding energy, kcal/mol | H-bonds between MHCs and ORF10 amino acid residues (res/length, Å) | Figure |
|-------|---|-------------------------|------------------------------------------------------------------|--------|
| Chlorin | 1 | −7.0                     | –                                                                | Fig. 4S |
| ClFePP | 1 | −6.1                     | ALA28/3.8* VAL30/2.0 ASP31/2.6 VAL32/2.4                         | Fig. 5S |
| PP    | 2 | −5.5                     | THR12/2.7* PHE9/2.2                                               | Fig. 6S |
|       | 1 | −5.9                     | VAL32/2.4 VAL30/2.4                                               | Fig. 7S |
|       | 2 | −5.9                     | ARG20/2.0                                                         | Fig. 8S |
|       | 3 | −5.8                     | ASN5/2.7**                                                        | Fig. 9S |
|       | 4 | −5.8                     | THR12/2.5**                                                       | Figure 10S |

* – the bond between metal ion and protein.

** – the bond between MHCs macroring and protein.

![Typical spectra of Chlorin titration with ORF10 protein is shown in Fig. 3. As can be seen from the presented spectra, an increase in the amount of protein leads to spectral changes. An isosbestic point is present on the UV–Vis titration spectra, which indicates the binding of Chlorin to the protein, however, no shift in the absorption bands of Chlorin was detected.](image)
As can be seen from this figure, the absorption of Chlorin changes significantly at the first dose of titration, and as the protein binds Chlorin, the changes decrease. The integral titration curve (Fig. 4, inset) has a characteristic inflection attributable to the molar composition of 1:1, reflecting the composition of the resulting complex.

The obtained spectral data were processed in the Scatchard approximation, a typical plot is shown in Fig. 5. The dependence is described by a straight-line equation with a correlation coefficient of 0.995, which indicates the formation of a single kind of complex with Chlorine. This conclusion confirms the results of molecular docking. The resulting affinity constants are presented in Table 2.

The study of the binding of ORF10 to protoporphyrin and iron(III)protoporphyrin is a more difficult experimental problem, due to the low solubility of these macrocycles. Adding the required amount of a solution of CFePP in DMF to sodium phosphate buffer leads to aggregation of CFePP and precipitation. The addition of the same amounts of MHCs to a solution of ORF10 in PBS does not lead to the formation of a precipitate, but the establishment of aggregation and complexing equilibria takes a very long time. As an example, Fig. 6 shows the UV–Vis spectrum of ORF10 (7.49 \times 10^{-4} \text{ M}) in sodium phosphate buffer (pH = 8.0) with the addition of 0.64 \mu l of a solution of protoporphyrin (1.63 \times 10^{-3} \text{ M}) in DMF over time. As can be seen from the spectrum obtained within 5 min after the addition of the porphyrin solution to the protein, the UV–Vis spectrum of the porphyrin is not resolved, which is due to the processes of self-aggregation of the porphyrin. The presence of protoporphyrin self-aggregation is confirmed by the absence of protoporphyrin fluorescence. After 12 h, the aggregation equilibrium shifts towards monomerization due to the binding of protoporphyrin by the protein, while the bands on the spectra become more pronounced, the obtained UV–Vis and fluorescence spectra of protoporphyrin are shown in Fig. 6. It will take more than 10 days to obtain quantitative parameters of the interaction of protoporphyrin with ORF10, but the "viability" of the ORF10 protein at room temperature is not known. In addition, it is impossible to obtain difference spectra due to the insolubility of porphyrins in buffer. Difficulties in interpreting the spectra of porphyrin complexes with ORF10 are also caused by MHCs aggregation processes that contribute to the spectral manifestation.

These features of the ORF10 systems with protoporphyrin and iron(III)protoporphyrin do not allow the use of traditional titration techniques, so we proposed a different approach to study these systems. The interaction of ORF10 with protoporphyrin and iron(III)protoporphyrin was studied against the background of a fluorescent marker, Chlorin. The fluorescence of Chlorin was excited by light with a wavelength of 425 nm, due to the active absorption of Chlorin in the region of 425 nm, the recorded fluorescence spectra were corrected taking into account light reabsorption. Fig. 7 shows the dependences of the change in the fluorescence of Chlorin in the PBS and in the PBS containing the CFePP complex with ORF10. As can be seen from the presented data, the fluorescence of Chlorin during competitive titration is lower than in the individual buffer. This agrees with the results presented above for the complex of chlorin with ORF10, according to which chlorin in the complex with protein fluoresces less. Thus, a 6-fold molar excess of chlorin, CFePP is completely displaced from its complex with ORF10 (Fig. 8). Similar dependences were obtained upon replacement titration of the PP complex with ORF10 with chlorine. In this case, a 4-fold molar excess of chlorine is sufficient for complete replacement of PP from the PP-ORF10 complex. The data obtained are consistent with the results of a theoretical assessment of the stability of the complexes (Table 1), according to which the affinity of ORF10 is higher for CFePP, compared with the affinity for PP, therefore, to displace CFePP from the complex with the protein, a larger amount of Chlorin is required than when PP is displaced.

Table 2

| MHCs  | Scatchard constants | The number of binding sites |
|-------|---------------------|---------------------------|
| Chlorin | 3.21 \times 10^{5} | 1.2                       |
| PP     | 2.68 \times 10^{4} | 1.6                       |
| CFePP  | 5.70 \times 10^{4} | 2.1                       |
The next part of the work was IR spectral studies of the ORF10 protein and its complexes with MHCs. The view of the IR spectrum of ORF10 in KBr is shown in Fig. 9.

It is known that the infrared spectra of proteins show a number of amide bands that correspond to different vibrations of the peptide fragment. The amide-I (1700–1600 cm\(^{-1}\)) and amide-III (1350–1200 cm\(^{-1}\)) regions are most often used to study the secondary structure of proteins, since the amide-II bands strongly overlap with those that arise due to vibrations in the side chains of amino acid residues. In the amide-I region, there are C = O stretching bands of the peptide group. The participation of this group in hydrogen bonding and dipole–dipole interactions with other fragments of the polypeptide chain leads to splitting of the vibrational band [39]. The magnitude of this splitting depends on the orientation and distance of the interacting dipoles and thus provides information on the geometric arrangements of peptide groups in the polypeptide chain [39]. In the first amide region, bands are fixed in the region of 1653, 1640 and 1619 cm\(^{-1}\) (Figure 11S).

These bands reflect the energy state of the C = O valence bond, depending on the effect on the C = O bond of protein regions that form various types of secondary structure (\(\alpha\)-, \(\beta\)- and disordered structures, respectively)[40].

In the spectral range of amide-III (1350–1200 cm\(^{-1}\)) (Figure 12S), three bands are fixed, very weak in the region of 1329 cm\(^{-1}\), weak at 1296 cm\(^{-1}\) and medium at 1255 cm\(^{-1}\). They correspond to the state of the N-H group in regions of proteins with different secondary structures. These bands are the result of in-plane bending of the N-H bond and stretching of the C-N bond. They are considered to be the most sensitive to the secondary structure of the protein [39,41]. The correlation of the spectral bands in the amide-III region is as follows: the band at 1330–1295 cm\(^{-1}\) corresponds to the \(\alpha\)-helix; band at 1295–1270 cm\(^{-1}\) - \(\beta\)-antiparallel sheets and/or \(\beta\)-turns; the bands at 1270–1250 cm\(^{-1}\) are of disordered structure, and the band at 1250–1220 cm\(^{-1}\) are \(\beta\)-parallel sheets. Based on the obtained data, it can be concluded that the secondary structure of ORF10 mainly consists of disordered fragments, \(\beta\)-antiparallel sheets, and \(\alpha\)-helix.

Thus obtained IR spectra of ORF10 confirm the presence of three types of secondary structures (\(\alpha\)-helices, and disordered structures, \(\beta\)-antiparallel sheets), previously proposed theoretically [42].

Figures 13S-15S show typical IR spectra of ORF10 complexes with protoporphyrin, iron(III)protoporphyrin, and Chlorin. The obtained spectra of ORF10 complexes with MHCs are not the sum of the spectra of the starting reagents. However, it should be noted that no new bands were found in the IR spectra of the complexes, but in some cases a change in the intensity of vibrational
modes and their shift were recorded, these spectral changes confirm the formation of complexes between MHCs and the protein.

Complexation of ORF10 with protoporphyrin (Figure 14S) manifests itself in a decrease in the intensity of the bands in the region of 1640, 1130, 997, 952, 864, 820, 762 cm\(^{-1}\). The binding of iron(III) protoporphyrin to the protein leads to a decrease in the intensity of the bands in the region of 1120, 1066, 952, 620 cm\(^{-1}\) and an increase in the intensity of the bands in the region of 1617 cm\(^{-1}\) and an increase in the intensity of most of the vibrational bands in the range of 1260–864 cm\(^{-1}\) and in the region of 616 and 514 cm\(^{-1}\).

In the case of protein binding to Chlorin, a decrease in the intensity of the bands in the region of 1617 cm\(^{-1}\) and an increase in the intensity of most of the vibrational bands in the range of 1260–864 cm\(^{-1}\) and in the region of 616 and 514 cm\(^{-1}\) were recorded. These spectral changes are consistent with the theoretically obtained interaction model, according to which, when protoporphyrin and iron(III)protoporphyrin are bound, axial and H-bonds are formed with the participation of atoms of the MHCs reaction center (Table 1). Therefore, a decrease in the intensity of the vibrational frequencies of the characteristic bands of the porphyrin ring (the region around 1400, 1120, 1170, 960, 620 cm\(^{-1}\)), symmetric bending vibrations of C-H pyrrole rings (1030–964 cm\(^{-1}\)) and bending vibrations of C-H aromatic fragments (1180–1060 cm\(^{-1}\)). Chlorine, in contrast to the studied porphyrins, does not form specific complexes with protein, the driving forces of the complex formation process are hydrophobic interaction, therefore, an increase in the intensity of most vibrational bands is recorded in the IR spectra.

4. Conclusions

In this study, the SARS-CoV-2 ORF10 protein and its interaction with protoporphyrin, iron(III) protoporphyrin, and Chlorin were theoretically and experimentally studied. It has been established that SARS-CoV-2 ORF10 protein has a higher affinity for Chlorin compared to porphyrins. It was revealed that the nature of the interaction of Chlorin with SARS-CoV-2 ORF10 is hydrophobic, specific bonds are not formed. This parameter is one of the main conditions for the use of Chlorine as a photosensitizer. Porphyrin forms several types of complexes with the ORF10 protein, localized in the regions of \(\alpha\)– and \(\beta\)-MoRFs formed due to hydrophobic and H-bonding between the porphyrin and amino acid residues of the protein.

**Author contributions**

N.L., Y.G. designed the project, wrote the manuscript with input from; Y.G., E.Y. performed investigations; A.M., Y.R., M.K. organized data; O.K. supervised the project. All authors read and approved the final manuscript.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary material**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2022.121403.

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