Aptamer BC 007 - Efficient binder of spreading-critical SARS-CoV-2 proteins

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ABSTRACT

Corona virus disease 2019 (COVID-19) is a respiratory disease caused by a new coronavirus (SARS-CoV-2) which causes significant morbidity and mortality. The emergence of this novel and highly pathogenic SARS-CoV-2 and its rapid international spread poses a serious global public health emergency. To date 32,174,627 cases, of which 962,613 (2.99%) have died, have been reported (https://www.who.int/westernpacific/health-topics/coronavirus, accessed 23 Sep 2020). The outbreak was declared a Public Health Emergency of International Concern on 30 January 2020. There are still not many SARS-CoV-2-specific and effective treatments or vaccines available. A second round of infection is obviously unavoidable.

Aptamers had already been at the centre of interest in the fight against viruses before now. The selection and development of a new aptamer is, however, a time-consuming process. We therefore checked whether a clinically developed aptamer, BC 007, which is currently in phase 2 of clinical testing for a different indication, would also be able to efficiently bind DNA-susceptible peptide structures from SARS-CoV-2-spreading crucial proteins, such as the receptor binding domain (RBD) of the spike protein and the RNA dependent RNA polymerase of SARS-CoV-2 (re-purposing). Indeed, several such sequence-sections have been identified. In particular for two of these sequences, BC 007 showed specific binding in a therapy-relevant concentration range, as shown in Nuclear magnetic resonance (NMR)- and Circular dicroism (CD)-spectroscopy and isothermal titration calorimetry (ITC). The excellent clinical toxicity and tolerability profile of this substance opens up an opportunity for rapid clinical testing of its COVID-19 effectiveness.

1. Introduction

The urgent need to identify therapy solutions to treat serious COVID-19 cases, alongside the vaccine development enables humans to look in every possible direction right now. SARS-CoV-2, a member of the Betacoronavirus family, “contains a positive-sense single-stranded RNA [(+)] ssRNA] genome (29,903 bp) and contains genes encoding 3C-like protease, RNA-dependent RNA polymerase (RdRp), 2′-O ribose methyltransferase, spike protein, envelope protein, nucleocapsid phosphoprotein, and others (http://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/)”; all of these are potential targets in the search for effective drugs [1].

With respect to drug development, it is anti-viral drugs, especially those which are known from former endeavours to fight against human immunodeficiency virus or other viral infections, targeting replication-critical proteins to interfere with viral replication such as protease inhibitors, reverse transcriptase or endonuclease inhibitors [1, 2]. An inhibitor of the RNA dependent RNA polymerase, Remdesivir [3], which was originally under development for the combat of Ebola has already been approved for its use in COVID-19.

Other therapeutic strategies besides the direct attack of the virus replication machinery are, however, also in the focus [2], including anti-inflammatoryary routes and virus entry inhibition via biologics (e.g. anti-sera). This list is certainly not complete and is growing daily, currently including 3352 entries in the clinical trial register “Clinical Trials.gov” in association with COVID-19; of these, 1918 entries are interventive studies (23 Sep 2020). Since only 238 entries are entitled
vaccine(s) development, all other interventive studies concentrate on identifying drugs or combinations of available drugs or drugs in existing pipelines ("re-purposing"). A direct inhibition of the virus entry by, e.g. therapeutic antibodies – the action principle of the anti-sera – is also part of the current endeavours [4, 5, 6]. Entry inhibition by therapeutic antibodies is a promising strategy, when looking at the therapeutic success which has been achieved with this new class of biologics over the last generation. However, therapeutic antibodies have well-known risks, as they may possibly be immunogenic.

We therefore returned to former endeavours to replace therapeutic antibodies with aptamers. Aptamers are non-immunogenic, when not modified, and can be produced in a large scale in short times. Aptamers have already been a substance class of interest in the search for antiviral therapy (for review see [7, 8, 9]); also already for anti-coronavirus therapy [10, 11]. Anti-viral aptamers showed a varying degree of success in vitro and in vivo. For the last group, the anti-influenza aptamer A22 blocking the receptor binding region of hemagglutinin has to be mentioned in particular [12].

A new aptamer selection – although a lot less laborious compared to the identification of therapeutic antibodies – would still, in the current stage of the pandemic, be too time-consuming. A re-purposing of existing aptamers for COVID-19 treatment has, therefore, most recently been suggested [13] and would be an elegant solution.

In addition to Wu et al. [14], Pokhrel et al. [15], Jiang et al. [16] and especially Kowalewski and Ray [17] among others, who used in silico methodologies to analyse which existing drugs would bind to known protein-structures of SARS-CoV-2, thereby identifying, besides known virustatic agents, an anti-ulcer drug, Famotidine, as a possible 3CLpro-blocker of SARS-CoV-2 [14] and several antibiotics as possible blockers of the RNA dependent RNA polymerase [15], we, here studied essential SARS-CoV-2 proteins for possible susceptibility to be attacked by a ssDNA-aptamer (BC 007).

In its current purpose BC 007 is able to neutralise pathogenic auto-antibodies [18, 19] as they occur in diseases of the heart and circulatory system [20] (for review see Dünge et al., 2020 [21]), and is already in phase II of the clinical testing (NCT04192214). In phase I (NCT02955420) it showed an excellent safety profile [22]. In the case of SARS-CoV-2 growth inhibition, re-purposing can be easily organised. This would be supported by the easy manufacturing and excellent drug stability.

In this study we identified several sequence-sections of spreading crucial SARS-CoV-2 proteins (spike protein and RNA dependent RNA polymerase) which are theoretically highly susceptible to interactions with DNA. In the following, the actual binding effect of these sequences with BC 007 was examined in more detail, exploiting NMR- and CD-spectroscopy and isothermal titration calorimetry (ITC). Using 3D models of these SARS-CoV-2 proteins, the BC 007-accessibility of the identified sites was checked.

2. Material and methods

2.1. Materials

BC 007 (5′-GGTTGGTGTGGTTGG-3′) was synthesised by BioSpring GmbH (Frankfurt, Germany) under GMP conditions. Peptides were synthesised by Biosyntan GmbH (Berlin-Buch, Germany) using solid phase synthesis with a purity >95% (MALDITOF; MALDI AXIMA Assurance, Shimadzu, Japan).

2.2. Methods

2.2.1. Nuclear magnetic resonance spectroscopy (NMR)

All NMR data investigating the interaction of BC 007 with sequence-sections of essential SARS-CoV-2 proteins were acquired at 600 MHz on a Bruker Av600 spectrometer (Bruker BioSpin, Rheinstetten, Germany) in 90/10 H2O/D2O at 298K (BC 007 at a final concentration of 1 mM and YRLFRK, HRFYRLAN, KIKRMK, KYRYLHRGKL, TNSPRARSV, SKPSKRSF, and LYRRNDV at final concentrations of 0.9 mM, 0.85 mM, 0.73 mM, 0.63 mM, 0.75 mM, 0.72 mM and 0.78 mM, respectively). The solvent signal was suppressed using the Watergate w5 pulse sequence included in the Bruker pulse program zgwpw5. Acquisition parameters included: time domain 65K, number of scans 512, sweep width = 24 ppm and 90° high power pulse = 13.8 μs.

2.2.2. High-sensitivity isothermal titration calorimetry (ITC)

ITC was used to characterise the binding affinity of BC 007 interaction with peptide sequence sections of growth crucial SARS-CoV-2 proteins. The experiments were performed on MicroCal PEAQ-ITC microcalorimeter (Malvern Panalytical GmbH, Germany). Both interaction partners were solved in 50 mM sodium phosphate, 150 mM NaCl buffer, pH 7.0-7.2 (7.06 for HRFYRLAN, 7.16 for NRKRISN and pH 7.19 for YRLFRK). Experiments were carried out at 25 °C.

In routine experiments, peptide (3.6 or 4 mM) was titrated in 2 μl-steps into aptamer solution (200 μM) in the calorimeter cell. Time intervals between the injections were adjusted to 200 s, which was sufficient for the heat signal to return to baseline. Reaction mixtures were continuously stirred at 750 rpm. For each peptide, all measurements were carried out twice and demonstrated high reproducibility. Dilution heats associated with the peptides addition into buffer (determined in separate control experiments) had small constant values that were negligible to measured binding heats. The instrument software (MicroCal PEAQ-ITC Analysis) was used for baseline adjustment, peak integration and normalization of the reaction heats with respect to the molar amount of injected ligand as well as for data fitting and binding parameter evaluation.

2.2.3. Circular dichroism-spectroscopy (CD)

The CD spectra of BC 007-peptide solutions, which were mostly taken from the ITC-measuring cell and diluted to a concentration of 21.5 μM, were recorded at 25 °C using a Jasco J-720 spectrometer equipped with a CD-Peltier Element Jasco PTC-423S/15 (JASCO, Japan) for temperature control.

Spectra were recorded at wavelengths in the range of 200–350 nm using the following recording conditions: cell length = 0.2 cm, scanning speed = 100 nm/min, response time = 1 s, band width = 1 nm, accumulation = 5 and data pitch = 0.1 nm. Prior to the experimental block, the CD spectrometer was calibrated using d-10-(+)-camphorsulphonic acid at 290 nm. The CD spectroscopy data measure differential absorption of left and right circularly polarised light and are reported as ellipticity [expressed in millidegrees (mdeg)]. The sample-corresponding buffer control spectra were subtracted.

3. Results and discussion

3.1. Identification and selection of DNA susceptible sequence-sections of SARS-CoV-2 proteins

In the spike protein of SARS-CoV-2, several sequences which are highly susceptible to interaction with DNA (multiple amino acids with positive charged side chains) were identified, in particular at the angiotensin I-converting enzyme 2 (ACE2)-receptor binding domain (RBD): YRLFRK (SARS-CoV-2 specific from protein data bank (PDB) data base entry PDB ID: 6VXX, source: [23]), as well as NRKRISN (PDB ID: 6VXX) and KIKRMK (PDB ID: 5X5B source: [24]). A different sequence, KKYRHYHKGL, was, however, identified from a SARS-CoV entry: PDB ID: 6ACJ, source: [25], enlarging the therapeutic anti-viral spectrum in the case of BC 007 binding effect.

Additionally, a peptide sequence from the multi-basic S1/S2 cleavage site of the spike protein of SARS-CoV-2, which is required for syncytium formation and entry into human lung cells (TNSPRARSV), as well as a sequence-section from the S2’ cleavage site (SKPSKRSF) [26] were identified as possible BC 007 binding candidates.
However, not only virus entry-mediating but also replication-mediating proteins seem to be promising targets in the fight against the virus. DNA-susceptible sequence sections from the RNA dependent RNA polymerase were, therefore, also included in this study: LYRNRDV and HRFYRLAN (PDB entry ID: 6M71, source [27]).

3.2. Characterisation of the BC 007-peptide binding using nuclear magnetic resonance spectroscopy (NMR)

From previous investigations with this aptamer, which was originally selected as a potential short-lasting thrombin inhibitor for transient anticoagulation during coronary bypass graft surgery [28] yet failed in this indication because of a lack of a persistent effect, resulting in a suboptimal dosing profile (too high a dose for a therapeutic effect), we learned that sections of the protein binding sequence (RTRYERNIE (from PDB ID entry: 1HAO, source [29] from the exosite 1) are already sufficient to enforce binding (data not shown). In this very special case, the formation of the quadruple structure of the aptamer is a very convenient readout of specific intermolecular interactions that can be easily detected via NMR spectroscopy [30] and CD-spectroscopy [31, 32]. In aqueous solution, BC 007 presents in a random coil structure. Only in the presence of alkali and alkaline earth metals, and especially the protein-binding partners or peptide sequences of those, BC 007 changes its structure into this well-defined quadruple appearance which presents in the NMR as a typical imino signal-pattern in the range from 11.5–12.5 ppm [31, 33] (see also Figure 1A green spectrum bottom).

This enabled us to exploit NMR-spectroscopy to investigate whether the selected peptide sequences from SARS-CoV-2 proteins bind to this clinically advanced aptamer (BC 007), forcing it into its well described quadruple structure just by molecular interaction.

This was indeed the case with a sequence of the ACE2-receptor binding domain YRLFRK (Figure 1A, B), while other sequences as NRKRISN and KIKRMK were a lot less effective (Figure 1C). The interaction of BC 007 with SARS-CoV-originated KYRYLRHGK was also able to force the molecule into the quadruple structure (Figure 1D).

Well selected sequence sections from the spike protein cleavage sites (TNSPRRSSV and SKPSKRSSF) and from the RNA dependent RNA polymerase (LYRNRDV and HRFYRLAN) also forced BC 007 into its quadruple structure, as shown in Figure 1D. Figure 1D shows, moreover, a ranking of the peptides, with respect to forcing BC 007 into its quadruple form. The two most effective sequences are the peptide HRFYRLAN derived from the RNA dependent RNA polymerase and the peptide YRLFRK derived from the RBD domain of the spike protein of SARS-CoV-2. Some of the BC 007 signals were significantly broadened. In particular, the H6 protons of the nucleotides T13 and T4, which belong to the TT loop of the folded BC 007, showed strong line broadening. The assignment of the BC 007 signals in both BC 007-peptide mixtures succeeded analogously to the assignment of the signals in the BC 007-potassium sample, and largely corresponded to the 1H-signal assignments known from the literature [32, 33].

In addition to the quadruplex formation of BC 007, the binding between BC 007 and the peptides also shows an extremely strong line broadening of the 1H-NMR proton signals on the peptide sides and strong chemical shifts compared to the free peptides (Figure 1B). Above all, the strong line broadening is unusual and leads to the absence of almost all expected cross peaks in the 2D NMR spectra.

Figure 1. NMR spectroscopic evaluation of the molecular interaction of BC 007 with sequence sections of the spike protein and the RNA dependent RNA polymerase of SARS-CoV-2. A: The upper NMR spectrum of BC 007 (1 mM) in combination with 0.9 mM YRLFRK (in blue colour) shows formation of the quadruplex structure of BC 007 induced by molecular interaction with the peptide which is clearly recognisable by eight imino signals between 11.5 and 12.5 ppm which are comparable to the well-known potassium-induced fold of BC 007 [32] (lower green spectrum). Substances were dissolved in 0.5 ml pure H2O/D2O mixture without any additives. The peptide signals in the spectrum are strongly shifted and broadened in comparison to the spectrum taken from the peptide alone without BC 007 (red colour). B: Same spectra zoomed into the range between 8.5 and 5.5 ppm. It shows that the peptide signals (red spectrum) are then broadened and shifted in their position in the upper spectrum (blue) due to the interaction between peptide and BC 007. C: BC 007 in presence of KIKRMK and NRKRISN showing an almost negligible intermolecular interaction. D: Ranking of the NMR spectroscopic evaluation of the molecular interaction of BC 007 with sequence sections of the ACE2-receptor binding domain (RBD) of SARS-CoV-2 (YRLFRK), of the S1/S2 (TNSPRRSSV) and S2 (SKPSKRSSF) cleavage sites of the spike protein, of the RNA dependent RNA polymerase (LYRNRDV and HRFYRLAN) and of the SARS-CoV spike protein (KRYRLHGK).
(Nuclear Overhauser Enhancement Spectroscopy (NOESY) (Figure 2A,B) and also Total Correlation Spectroscopy (TOCSY) (Figure 2C), so that even the simple assignment of proton signals to the individual amino acids proves to be difficult or impossible. Only a few spin systems are completely recognisable in the TOCSY spectra. Attempts to reduce the signal broadening were made by raising and lowering the temperature. Even at temperatures of 5 °C and 40 °C, the spectra cannot be sufficiently improved (data not shown). Extension of the mixing time in the TOCSY and NOESY spectra also fails to reveal the "missing" peptide signals.

The NOESY spectrum shows in addition to the NOE signals from BC 007 also numerous exchange peaks of folded and unfolded BC 007 in the

Figure 2. 2D NMR spectroscopic evaluation of the YRLFRK-BC 007 binding. Comparison of the NOESY spectra between A: BC 007-YRLFRK and B: BC 007-K⁺, both recorded in H₂O/D₂O 90/10 at 298K. Dotted lines enclose the binding relevant residues T3/T4 and T12/T13 of the TT-loops. C: ¹H-TOCSY spectrum (600 MHz) of the 1mM mixture BC 007-YRLFRK at 298K in H₂O/D₂O 90/10. Pulse program mlevgpphw5, size of fid 4096 × 512, number of scans 64, spectral width 24 ppm, TOCSY mixing time 70 ms.
NH/NH region of the amino acids and in the Hb/Hh region of the NOESY spectra of the two samples. The presence of BC 007 random coil signals is due to a slight excess of BC 007 in the mixtures (see under Material and methods, NMR) explaining the associated incomplete folding of the aptamer.

The strong line broadening and the resulting lack of important cross peaks in the NOESY (and also TOCSY) spectra prevent molecular dynamics simulations using NMR constraints. At the same time, the strong similarities between $^1$H-NMR shifts of the BC 007-peptide samples and the potassium-induced structure (Table 1) allows the conclusion that potassium- and peptide-induced BC 007 quadruplex structures are very similar.

Noticeable deviations exist for the H6-protons of nucleotides T3, T4 and T12, T13 and for the H8 proton of G8 (Table 1). The thymine residues formed two TT loops in presence of the peptides, which have already been described for the binding of the aptamer with thrombin [30].

The prominent position of guanine 8 also offers opportunities for interacting with amino acid side chains, especially arginine and lysine.

### 3.3. Estimation of the BC 007-peptide dissociation constant (K_d) using isothermal titration calorimetry (ITC)

Broading the proton signals in the $^1$H-NMR spectra upon interaction of BC 007 and peptides is an indication of the dynamic binding process with dissociation constants in the μM-range. Therefore, isothermal titration calorimetry (ITC) was used to investigate the affinity of three selected peptide sequences taken from the NMR-ranking list (hit no. 1: HRFYRLAN from the RNA dependent RNA polymerase, hit no. 2: YRLFRK from the ACE2-receptor binding domain of the spike protein) and one even though physicochemically similar, but in the NMR non-binding peptide sequence (NRKRISN) for comparison (Figure 3).

ITC excellently confirmed the NMR data fully reflecting the ranking. While HRFYRLAN showed the highest affinity with $K_d = 54.7$ μM, followed by YRLFRK with $K_d = 86.7$ μM, NRKRISN did not show any specific binding (Figure 3). Full thermodynamic profiles of the hit peptides-BC 007 binding are presented in Table 2.

Both HRFYRLAN and YRLFRK show similar interaction patterns: binding is strongly exothermic with large favorable enthalpy changes (Δ$H^0$) that overcompensate large unfavorable entropic contribution (-TΔS°). The values of Δ$H^0$ and -TΔS° show that in both cases the binding processes are solely enthalpically driven. The stoichiometry of both complexes N = 1 clearly indicated that BC 007 poses one binding site for the peptide ligands. The values of the Gibb free energy changes (Δ$G^0$) indicate that the binding of HRFYRLAN and YRLFRK with aptamer is strongly thermodynamically favored. Although the difference in Δ$G^0$ between two peptides is not so large with Δ$G^0$ = -1.2 kJ/mol, it gives clearly measurable difference in the binding affinity. HRFYRLAN with $K_d$ of 54.7 μM binds BC 007 1.6 times stronger than the YRLFRK with $K_d$ of 86.7 μM.

Negative values of the reaction entropy changes (Table 2) are most likely attributed to the structural rearrangement due to folding of BC 007 molecules upon peptides’ binding. The folding process was reported previously [30] and verified in the current study by CD spectroscopy (see under section 3.4). Entropic loss of the energy was compensated by the strong enthalpic gain that most likely attributed to the formation of new hydrogen bonding and salt bridges in the peptide-BC 007 complexes.

ITC revealed a true binding, resulting in a binding stoichiometry of 1:1 for BC 007-HRFYRLAN (HRFYRLAN theoretical pl value of 10.84, ExPASy [34] and BC 007-YRLFRK (YRLFRK theoretical pl value of 11.0, ExPASy)), excluding simple electrostatic attraction. This was supported by the fact that the physicochemically similar NRKRISN (theoretical pl value of 12.01, ExPASy) did not show any binding at all.

The comparably high $K_d$ values in the mid μM range (55–87 μM) is owed to the fact that these short peptide sequences were taken out from the entire protein. It is a known fact that the binding with larger proteins is additionally supported by hydrogen bonds and solvation desolvolation contributions [35].

### 3.4. Characterisation of the BC 007-peptide binding using circular dichroism spectroscopy (CD)

Using CD-spectroscopy, the folding success of the resulting final BC 007-peptide mixtures from the ITC experiment was re-checked and compared to each other (Figure 4).

Even though the RNA dependent RNA polymerase occurring peptide sequence, HRFYRLAN, showed the highest amount of folded BC 007, the maximal folding capacity was subsequently induced simply by adding 5 mM KCl to the peptide-aptamer mixture (Figure 5A).

The addition of 5 mM KCl enforced the complete folding of BC 007. At the positive peak of 295 nm (at this wavelength no influence of the peptides on the BC 007 spectrum has to be expected), the ratio between

| Table 1. The guanine-G8/thymine-H6 signal assignments of BC 007 in the presence of the SARS-CoV-2-derived peptides YRLFRK and HRFYRLAN and potassium ions. The potassium data serve for folding control; data were normalised to the H2O-signal. |
|-----------------|------------------|-----------------|
| BC 007          | K⁺ ion | YRLFRK | Peptides | HRFYRLAN |
| G1              | 7.42   | 7.37 | 7.38 |
| G2              | 8.17   | 8.17 | 8.16 |
| T3              | 7.86   | 7.92 | 7.58 |
| T4              | 7.19   | 7.08 | 7.09 |
| G5              | 7.43   | 7.40 | 7.01 |
| G6              | 7.69   | 7.67 | 7.41 |
| T7              | 7.90   | 7.88 | 7.71 |
| G8              | 7.48   | 7.61 | 7.89 |
| T9              | 7.26   | 7.22 | 7.87 |
| G10             | 7.46   | 7.43 | 7.77 |
| G11             | 8.19   | 8.17 | 7.77 |
| T12             | 7.86   | 7.91 | 7.45 |
| T13             | 7.24   | 7.14 | 7.65 |
| G14             | 7.48   | 7.45 | 7.11 |
| G15             | 8.06   | 8.02 | 7.46 |

Numbers in bold mark the largest differences of the observed $^1$H-NMR shifts of the peptide-induced BC 007 quadruplex structures from the K⁺-induced quadruplex structure which are found in the T3, T4, T12, T13 and G8 regions.
KCl-free and KCl-containing spectra was taken, which revealed the 87.8% folding of HRFYRLAN, 84% of YRLFRK and 26.5% of NRKRISN. However, it is not possible to see from those data whether the ratios fully correspond to the peptide-mediated folding success or also have other additional steric causes.

The BC 007 control spectrum in the diluted NaCl/sodium phosphate buffer shows the small impact of NaCl on folding. Avoiding any overlapping influence of salt on the folding of BC 007, NMR-spectroscopy was carried out in water. Water is, however, not appropriate for ITC measurements. Here, a buffered salt solution is strongly recommended. Since BC 007 also folds in the presence of Na\(^+\) ions – although a lot less compared to K\(^+\) [32] – the contribution of sodium ions to folding was controlled in this way and was about 20% (also compare to the 26.5% caused by NRKRISN). Even in 154 mM Na\(^+\), the folding capacity only reaches about 30% of the maximal folding effect [32]. Therefore, sodium did not have any essential influence on the CD-spectroscopic BC 007-peptide binding evaluation. ITC is not influenced by the buffer in any way, since only the actual effect of the binding process is monitored – with both binding partners dissolved in the same buffer.

Finally, the accessibility of the identified binding sites integrated in the entire protein for BC 007 binding was checked, using the 3D visualization of the protein data bank entries of the full proteins (PDB ID: 6M71 for HRFYRLAN and LYRNRDV of the RNA dependent RNA polymerase [27]; PDB ID: 6VXX for YRLFRK of the spike protein [23]). All essential sequences in the spike protein and the RNA dependent RNA polymerase are located on the surface of the proteins enabling a contact with BC 007, as exemplarily visualized for HRFYRLAN of the RNA dependent RNA polymerase in Figure 6A. The possible BC 007-HRFYRLAN binding in Figure 6 A shows a strong similarity to the well-investigated BC 007-thrombin binding (PDB ID: 1HAO [29], Figure 6B).

Just using an existing developed aptamer in an “trial and error set-up”, might, especially in view of the medical urgency, also lead to valuable

Table 2. Thermodynamic parameters of hit peptides binding to BC 007*.

| Peptide     | \(K_d\) (\(\mu\text{M}\)) | \(N\) | \(\Delta H^0\) (kJ/mol) | \(\Delta G^0\) (kJ/mol) | \(-T\Delta S^0\) (kJ/mol K) | \(\Delta S^0\) (kJ/mol*K) |
|-------------|--------------------------|------|-------------------------|-------------------------|----------------------------|---------------------------|
| HRFYRLAN    | 54.7 ± 0.6               | 1.01 | -108 ± 0.4              | -24.4                   | 83.8                       | -0.28                     |
| YRLFRK      | 86.7 ± 1.2               | 1.01 | -103 ± 0.7              | -23.2                   | 79.3                       | -0.27                     |

* Dissociation constant \(K_d\), enthalpy changes \(\Delta H^0\), and stoichiometry \(N\) are directly measured parameters, which are determined from the fit of binding isotherm. The error is the fitting error, showing how good the fit match the experimental data. Simple binding model “one set of sites” was used. Gibbs free energy changes, \(\Delta G^0\); entropy changes \(\Delta S^0\); temperature \(T\).
medicines that could be used very quickly in the combat against SARS-CoV-2. BC 007 had already undergone all preclinical and clinical toxicity and tolerability tests, without showing any toxicity, as well as an expected application accompanying anti-coagulatory effect [22]. The latter would, in view of the increased risk of COVID-19 associated thromboembolic events, be of additional advantage. In addition, if sufficient in reducing the virus load in vivo, BC 007 could have a useful contribution in the fight against COVID-19.

As its actual purpose is to neutralise pathogenic autoantibodies against G-protein coupled receptors [18] as they especially occur in diseases of the heart and circulatory system (for review see (Düngen et al., 2020)) and because it is currently in phase II of the clinical testing (NCT04192214), BC 007, is being given via slow infusion over e.g. one hour, circumventing its short in vivo half-life of 3–11 min (NCT02955420) [22]. In this way, the pharmacokinetics can excellently be controlled in the clinic, which is a fact that would also be of great benefit in the case of COVID-19 use.

4. Conclusion

Taking all of the BC 007-SARS-CoV-2 binding data together, showing true and efficient binding with peptide sequences of the RNA dependent RNA polymerase and the RBD of the spike protein, it can be concluded that it is highly probable that BC 007 will be able to efficiently interfere with the virus growth in vivo. The excellent safety profile of BC 007 should support a very early clinical testing in humans.

Declarations

Author contribution statement

A. Haberland: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
H. Weisshoff and O. Krylova: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
H. Nikolenko: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
H.D. Düngen: Conceived and designed the experiments; Analyzed and interpreted the data.
A. Dallmann: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
S. Becker: Analyzed and interpreted the data.
P. Götzel: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
J. Müller: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
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Declaration of interest statement
S. Becker, P. Göttel, A. Haberland, J. Müller are employed by Berlin Cures. P. Göttel, A. Haberland, J. Müller are shareholders of Berlin Cures AG. H. Weisshoff is employed by a co-operation project between the Humboldt-Innovation GmbH and Berlin Cures GmbH. A patent had been filed at the European Patent Office (no. 20 168 929.6) by the Berlin Cures GmbH.

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