The SARS-CoV and SARS-CoV-2 spike protein interactions with the human protein receptor ACE2 and the impact of zinc ions on that interaction

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Introduction

Coronaviruses are enveloped, large, moderately pleomorphic, positive-stranded RNA (+ssRNA) viruses belonging to the family Coronaviridae. They are divided into four major genera: Alphacoronaviruses, Betacoronaviruses, Gammacoronaviruses, and Deltacoronavirusea. Both severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) belong to the genus Betacoronavirus. SARS-CoV-1 (responsible for the SARS epidemic in 2002-2004) and SARS-CoV-2 (responsible for COVID -19) share many similarities. Both viruses cause respiratory diseases transmitted by contact with infected individuals. The genomes of SARS-CoV-1 and SARS-CoV-2 have 79.5% sequence identity and encode the non-structural replicase polyprotein, four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), and several additional non-structural proteins called accessory proteins

The spike protein (150 kDa) is a highly glycosylated homotrimer that is distributed on the surface of the virion particles and protrudes radially from the viral envelope, forming a "crown-like" structure. The S-glycoprotein is responsible for the binding of the virion to the host receptor, its fusion with it and entry into the virus. The SARS-CoV-1 and SARS-CoV-2 use angiotensin-converting enzyme 2 (ACE2), an enzyme found on the outer surface of a variety of cells, as their cellular receptor.

Each coronavirus spike protein consists of three segments: an ectodomain, a transmembrane anchor and an intracellular tail. Two subunits can be distinguished in the ectodomain of the S protein. The amino-terminal subunit (S1) is responsible for the binding of the virus to the ACE2 receptor, while the carboxyl-terminal subunit (S2) is responsible for the fusion of the virion with the cell membrane. The S1 subunit of coronaviruses includes the N-terminal domain (NTD), the receptor-binding domain (RBD) and two subdomains SD1 and SD2. The RBD contains a core and an extended loop called the receptor-binding motif (RBM), which interacts directly with ACE2 [1-7].

The aim of this study was the therodynamic analysis of the interactions between the human ACE2 receptor protein and RBDs of SARS-CoV and SARS-CoV-2 protein.

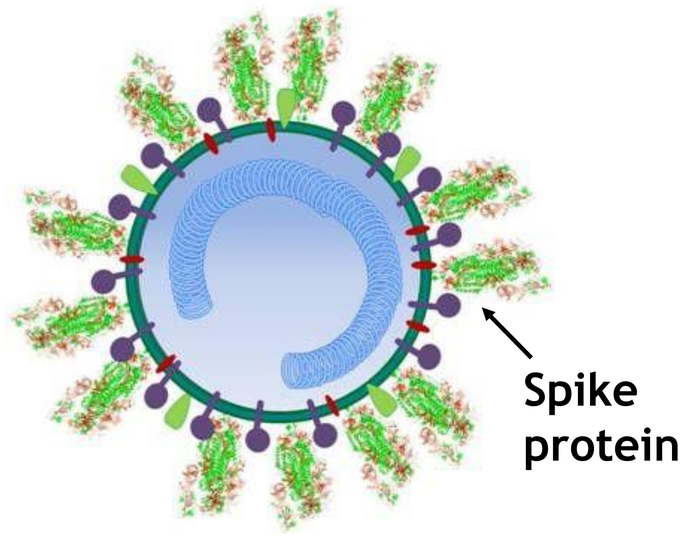

Fig. 1. The SARS-CoV-2 virion.

Methods

After exhaustive dialysis against PBS buffer, pH 7.4, the interactions of RBD^{CoV1} and RBD^{CoV2} with the hACE2 receptor were investigated using isothermal titration calorimetry (ITC). For each ITC assay, the RBDs and hACE2 receptor protein were dialyzed against the same buffer and during the same time period (the buffer was exchanged 4-5 times every 12 h) to ensure that all samples were as pure as possible and fit into the correct buffer to avoid heat changes due to buffer mismatch.

Isothermal titration calorimetry (ITC) measurements were performed at 25 °C and pH 7.4 on a MicroCal PEAQ Isothermal Titration Calorimeter. After the instrument was stabilized at 25 °C, 40 mL of RBD^{CoV1}, RBD^{CoV2} or RBD^{CoV2b} buffered solutions were used to titrate 200 mL of ACE2 buffered solutions (concentration initially approx. ten times lower than that of RBD) by 19 consecutive injections with an interval of 150 s between each drop and a stirring speed of 750 rpm (each test was repeated a few times). The reference cell was filled with distilled water.

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SARS-CoV-1        306  RVVPSGDVVRFP 317
SARS-CoV-2        319  RVQPTESIVRFP 330
SARS-CoV-2 SD1    319  RVQPTESIVRFP 330

SARS-CoV-1        318  NITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNV 382
SARS-CoV-2        331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV 395
SARS-CoV-2 SD1    331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV 395

SARS-CoV-1        383  YADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGK 447
SARS-CoV-2        396  YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN 460
SARS-CoV-2 SD1    396  YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN 460

SARS-CoV-1        448  LRPFERDISNVPFSPDGKPCT-PPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATV  510
SARS-CoV-2        461  LKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATV  524
SARS-CoV-2 SD1    461  LKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATV  524

SARS-CoV-1        511  CGPKLSTDLIKNQCVNF  527
SARS-CoV-2        525  CGPKKSTNLVKNKCVNF  541
SARS-CoV-2 SD1    525  CGPKKSTNLVKNKCVNF  541

SARS-CoV-2 SD1    542  XFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS●●●          591
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Fig 2. Sequence alignment of SARS-CoV-1 S1 RBD, SARS-CoV-2 S1 RBD and SARS-CoV-2 S1 RBD-SD1, named within this work: RBD^{CoV1}, RBD^{CoV2}, and RBD^{CoV2b}, respectively. Residues composing RBD are in magenta. Variable amino acid residues between SARS-CoV-1 and SARS-CoV-2 RBDs are in cyan.

Results

Both receptor binding domains (of SARS-CoV-1 and SARS-CoV-2) bind the hACE2 protein receptor with similar and high affinity and with a stoichiometry (N_{ITC}) = 1. Difference can be seen in the ΔH_{ITC} and ΔS_{ITC} values. When ACE2 interacts with RBD^{CoV2}, both the enthalpic contribution (ΔH_{ITC}) and entropic penalty are larger than when it interacts with RBD^{CoV1}. The enthalpy gain of the RBD^{CoV2} interaction is largely compensated by an entropy loss, resulting in no difference in affinity (K_{dITC}).

Table 1. Differences in the hACE2 receptor binding thermodynamics between RBD of SARS-CoV-1 and RBD of SARS-CoV-2.

	ACE2-RBD^{CoV1}	ACE2-RBD^{CoV2}
$K_{d\,ITC}$ [nM]	145.5 ± 25.0	144.0 ± 35.3
ΔH_{ITC} [kcal/mol]	-10.50 ± 0.27	-16.15 ± 0.60
N_{ITC}	1.03 ± 0.01	0.99 ± 0.02
$-T_{\Delta}S_{ITC}$ [kcal/mol]	1.15	6.81

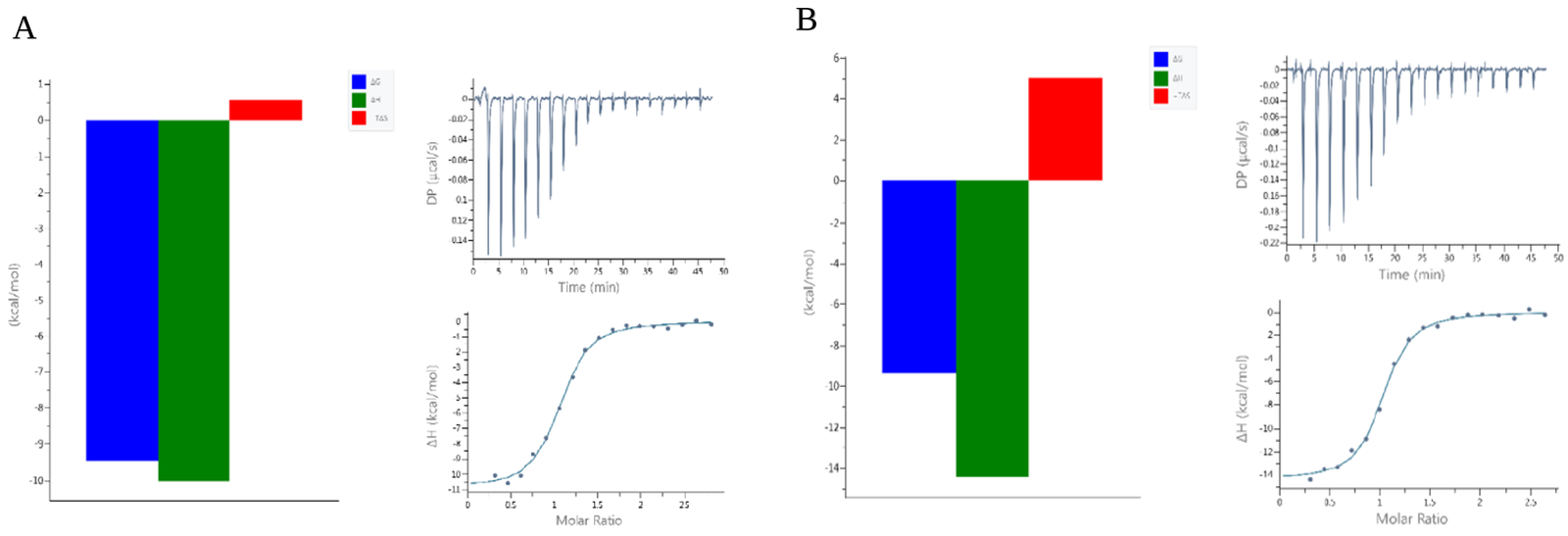

Fig. 3. Signatures (left panels) and calorimetric titration isotherms (right panels) of binding of (A) SARS-CoV-1 RBD and (B) SARS-CoV-2 RBD to the hACE2 receptor protein under the same experimental conditions (PBS buffer pH 7.4, 25 °C). The concentration of hACE2 was 6 µM and the concentration of the RBDs was in the range of 88-91 µM.

Large entropic penalties are observed for both systems, especially for the ACE2-RBD^{CoV2b} interactionThe results show that the zinc ions present in the buffer have no positive impact on RBD-ACE2.

Table 2. Binding of the ACE2 protein receptor by RBD of SARS-CoV-2, after dialysis of both binding partners in TRIS buffer containing 1 mM Zn^{2+} and without Zn^{2+}.

	ACE2 - RBD^{CoV2b} (buffer with Zn^{2+} ions)	ACE2 - RBD^{CoV2b} (buffer without Zn^{2+} ions)
$K_{d\,ITC}$ [nM]	17.9 ± 9.4	15.9 ± 2.3
ΔH_{ITC} [kcal/mol]	-15.2 ± 0.8	-22.15 ± 0.25
N_{ITC}	0.79 ± 0.01	0.83 ± 0.04
$-T_{\Delta}S_{ITC}$ [kcal/mol]	4.61	11.4

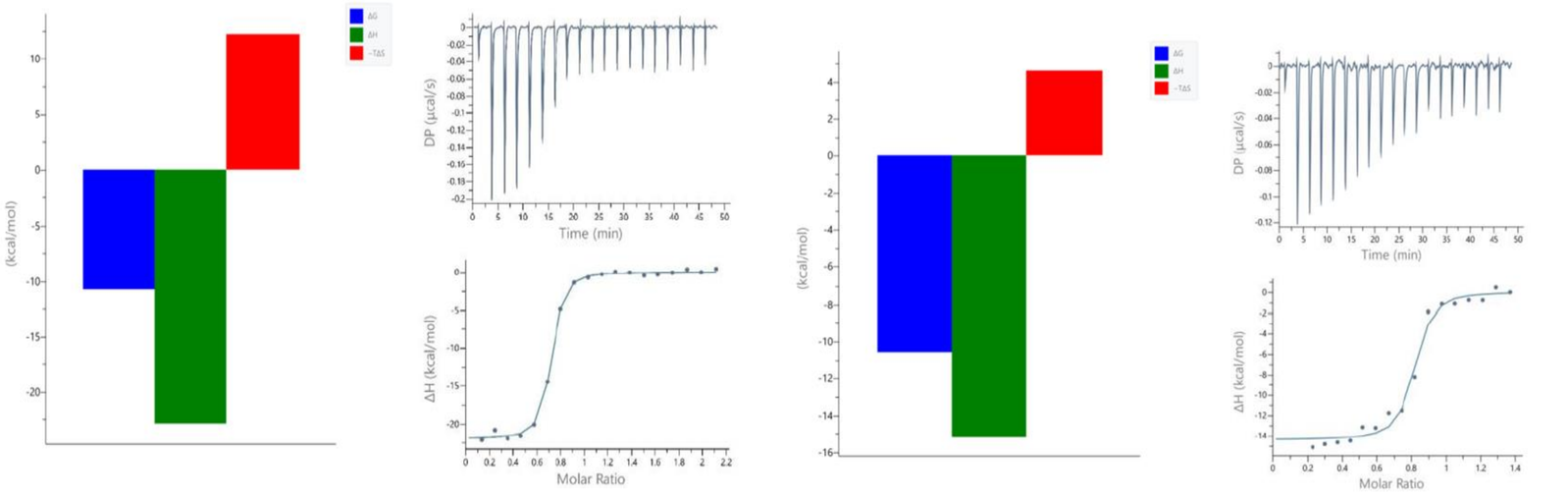

Fig. 4 Signatures (left panels) and calorimetric titration isotherms (right panels) of the binding of RBD^{CoV2b} to the hACE2 receptor in buffer with the addition of 1 mM $ZnCl_2$ (A) and without Zn^{2+} ions (B). The concentration of hACE2 was 4.5 µM and the concentration of the RBD was in the range of 33-55 µM.

References

[1] J. Shang, G. Ye, K. Shi, Y. Wan, C. Luo, H. Aihara, et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature*, 581:221-4 (2020).
[2] W. Song, M. Gui, X. Wang, Y. Xiang. Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cel receptor ACE2. *Plos Pathog*, 14:e1007236 (2018).
[3] W. Cao, C. Dong, S. Kim, D. Hou, W. Tai, L Du, et al. Biomechanical characterization of SARS-CoV-2 spike RBD and human ACE2 protein-protein interaction. *Biophys J*, 120:1011-19 (2021).
[4] J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature, 581, 215-20 (2020).
[5] M. Barton, S. MacGowan, M. Kutuzov, O. Dushek, G. Barton, P. Merwe. Effects of common mutations in the SARS-CoV-2 Spike RBD and its ligand, the human ACE2 receptor on binding affinity and kinetics. eLife, 10:e70658 (2021).
[6] G. Park, Hwang B. SARS-CoV-2 Variants: Mutations and Effective Changes. Biotechnology and Bioprocess Engineering, DOI 10.1007/s12257-021-0327-3 (2021).
[7] W. Zhou, W. Wang. Fast-spreading SARS-CoV-2 variants: challenges to and new design strategies of COVID-19 vaccines. Signal Transduction and Targeted Therapy, 6:226 (2021).

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