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Saliva glycoproteins bind to Spike protein of SARS-CoV-2

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Abstract

We analyzed the affinity-proteomics data of saliva absorbed to plate-bound Spike protein of SARS-CoV-2, and identified major virus-binding proteins as MUC7, MUC5B, DMBT1, and neutrophil defensins. Furthermore, we found that saliva from healthy donors inhibited the binding of Spike-protein-specific polyclonal antibodies to Spike antigen. These data suggest that the Spike protein's glycoprotein-binding domains (GBD) may be targeted to block virus adherence or entry of SARS-CoV-2.

Key words: SARS-CoV2; corona virus; affinity proteomics; glycoproteins; glycoprotein-binding domains

Introduction

Saliva is a major route of spreading for COVID-19 pandemic [1]. SARS-CoV-2 has been found in saliva of infected patients, with a virus titer of up to 10^8 PFU per ml. Multiple protein components such as MUC5B, defensins and DMBT1 have been reported to bind to viruses [2-4]. DMBT1 binds to gp120 of HIV-1 virus and mediates the viral infection in macrophages at mucosal site [5].

In this study, we performed affinity-proteomics analysis on saliva using recombinant SARS-CoV-2 Spike protein. We also examined the inhibition effect of saliva on polyclonal antibodies raised against Spike protein of SARS-CoV-2.

Results

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Spike protein of SARS-CoV-2 binds to multiple saliva proteins

A total of 138 proteins with ion abundance higher than 10^7 were absorbed by plate-bound Spike-protein from 3 volunteers (Table 1 and supplemental Tables 1-3). The proteins with highest ion abundance were MUC7, MUC5B, DMBT1, and neutrophil defensins. Most high-abundance saliva proteins in previous proteomics studies were not present in our samples absorbed by Spike protein [6-7], indicating that most saliva proteins do not bind to Spike protein. SDS-PAGE analysis of saliva samples before and after Spike protein absorption clearly showed that most salvia proteins were unbound and washed away, as visualized by silver staining (Supplemental Figure 1).

Saliva proteins inhibit the binding of polyclonal antibodies to Spike protein

To test whether saliva proteins inhibit the binding of polyclonal antibodies specific to Spike protein of SARS-CoV-2, we collected saliva of healthy donors of different gender and age groups (study protocol: 2020tjdx055, Tongji University). The inhibition rate was defined as the percentage of maximum inhibition in serial diluted antibody sample pooled from Spike protein-vaccinated mice. We found above 50% inhibition rate in 13 of the 22 donors (Figure 1). No significant difference were found among different age or gender groups. The maximum inhibition was observed at the sera dilution fold of 1:6075 to 1:54675 (Table 2 and Supplemental Figure 2), indicating that the vaccination-induced antibodies may overcome the inhibitory effect of saliva very efficiently.

Discussion

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Saliva proteins are known to contain virus-binding proteins and be involved in viral pathogenesis. Many viruses bind to saliva through recognizing glycan ligands attached to host mucins [8-12]. Sialic acid-containing glycoproteins are one of the critical receptors for cellular entry by several corona viruses including Transmissible gastroenteritis virus (TGEV) [13], porcine epidemic diarrhea virus (PEDV) [14], HCoV-OC43 and MERS [15-17]. It remains to be studied whether Spike protein of SARS-CoV-2 binds to glycans attached to host glycoproteins which we have discovered (Table 1).

Sugar binding domain of PEDV-CoV and MERS-CoV Spike protein is considered as important for the critical entry functions [13, 18]. Four monoclonal antibodies (MAbs 56, 60, 63, and 72) targeting the sialic acid-binding domain could neutralize GDU strain of PEDV-CoV virus at 0.015 to 0.039 μg/ml of concentration [13]. Passive immunization of mice with a mAb 1.10f3 which target sialic acid-binding domain of MERS-CoV resulted in 40% protection from mortality following MERS-CoV infection [18]. In our study, we observed the maximum inhibition rate of saliva protein in competing with the antibody binding to Spike protein at the titer of 1:6075 to 1:54675. The exact nature of the domains that bind to saliva glycoproteins and whether they are targets are neutralizing antibodies remain to be studied. Ongoing studies are being focused to isolate potential neutralizing antibodies in SARS-CoV-2 infected or vaccinated individuals that may block the Spike protein's glycoprotein-binding domains (GBD).

In summary, our study clearly identified saliva glycoproteins as binders of Spike protein of SARS-CoV-2. Glycoprotein-binding domains of SARS-CoV-2 virus are potential targets for the development of antibody therapeutics and vaccines.

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Methods

Affinity proteomics by recombinant Spike protein

Recombinant insect cell-derived Spike proteins for SARS-CoV-2 (GenBank Accession Number: MN908947) was prepared as described [19]. A plate-bound assay was used to purify saliva proteins which bind to Spike protein. 96-well Clear Flat Bottom Polystyrene High Bind Microplate (Corning) was coated by 1 mg/ml Spike protein in PBS at 4ºC for overnight, and washed five times with 0.05% Tween in PBS (every time for 2 minutes using a mini shaker). The S protein-coated plates were blocked with 1% bovine serum albumin (Sigma) at 37ºC for one hour, and washed five times with 0.05% Tween in PBS. Saliva were added at 50 µl per well and 96 wells were used to absorb saliva from every donor. The saliva samples were incubated for one hour at 37ºC to bind plate-bound S protein. Unbound saliva proteins were discarded and the plates were washed for five times with 0.05% Tween in PBS. Saliva proteins bound to S protein were released by adding 0.1 M Glycine-HCl (pH 2.5). The eluted proteins were neutralized by 50 mM Tris buffer (pH 8.0) and desalted by Pierce concentrator (30K, MWCO, 0.5 ml). The proteins samples were reduced and digested by trypsin (Promega) as described (19). The trypsin-digested peptides were desalted with mono-Spin C18 column (GL Sciences). The desalted peptide mixture were analyzed by LC-MS-MS as described using an Easy-nLC 1000 system (Thermo Scientific, San Jose, CA) and orbitrap analyzer (Q Exactive mass spectrometer, Thermo Scientific, San Jose, CA).

Preparation of anti-sera to Spike protein

All animal studies were approved by the animal care and use committee of Tongji University. All experiments were carried in SPF housing facilities. C57/BL6 strain of mice (male, 8 weeks

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old) were immunized by recombinant S protein with polyI:C as adjuvant [20]. 5 μg of S protein mixed with 50 μg of polyI:C adjuvant (Yisheng Biopharma, Beijing) were injected per mouse intramuscularly [20]. Two vaccination schedule were tested. In Schedule A, mice were immunized at days 0, 7 and 16. In Schedule B, mice were immunized at days 0, 3 and 7. Sera from immunized mice were collected by tail vein bleeding, and anti-S protein antibody titer was measured by ELISA. 50 μl of 1 μg/ml S protein in PBS was bound to 96-well Clear Flat Bottom Polystyrene High Bind Microplate (Corning) at 4ºC for overnight, and washed five times with 0.05% Tween in PBS (every time for 2 minutes using a mini shaker). The S protein-coated plates were blocked with 100 μl 1% bovine serum albumin at 37ºC for one hour, and washed five times with 0.05% Tween in PBS, followed by incubation with 50 μl serially diluted sera for one hour. The plates were washed for five times, and the anti-S protein mouse IgG was visualized by a secondary antibody (goat anti-mouse IgG, Southern Biotech) followed by colorimetric detection. One percent bovine serum albumin in PBS was used as blank to determine the cutoff value.

Measurement of inhibition rate of saliva on antibodies generated against S protein

Saliva of healthy donors of different gender and age groups were collected to test their inhibition rate on pooled mouse sera containing polyclonal anti-Spike protein antibodies (study protocol: 2020tjdx055, Tongji University). Saliva samples were directly used to block the plate-bound Spike protein for one hour before ELISA experiment as below: 50 μl of 1 μg/ml S protein was bound to 96-well Clear Flat Bottom Polystyrene High Bind Microplate (Corning) at 4ºC for overnight, and washed five times with 0.05% Tween in PBS (every time for 2 minutes using a mini shaker). The S protein-coated plates were blocked with 100 μl 1% bovine serum albumin at

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37ºC for one hour, and washed five times with 0.05% Tween in PBS. The plates were incubated with 50 µl saliva per well at 37 ºC for one hour, and washed for five times. Pooled sera from vaccinated mice (with anti-S antibody titer of 1:54675) were serially diluted and added, and anti-S protein mouse IgG was visualized by a secondary antibody (goat anti-mouse IgG, Southern Biotech) followed by colorimetric detection. The inhibition rate was determined as the percentage inhibition of absorbance at OD450, at the antibody dilution that showed maximum rate of inhibition.

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Conflict of interest disclosures

The authors declare no conflict of interest.

Author contributions

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Dapeng Zhou designed this study. Dapeng Zhou and Chenghao Wu contributed to the collection, analysis and interpretation of data. Dapeng Zhou wrote the manuscript. All authors read and approved the final manuscript.

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Figure legends:

Figure 1. Inhibition rate of saliva from healthy donors on the binding of Spike protein to polyclonal antibodies. Saliva samples were directly used to block the plate-bound Spike protein for one hour before ELISA experiment. The inhibition rate was determined as the percentage inhibition of absorbance at OD450, at the antibody dilution that showed maximum rate of inhibition (Table 2, Supplemental Figure 2).

Supplemental Online Materials

Supplemental Table 1: List of proteins absorbed by plate-bound Spike protein from saliva of volunteer A.

Supplemental Table 2: List of proteins absorbed by plate-bound Spike protein from saliva of volunteer B.

Supplemental Table 3: List of proteins absorbed by plate-bound Spike protein from saliva of volunteer C.

Supplemental Figure 1: SDS-PAGE of saliva proteins from healthy volunteers before and after absorption by plate-bound Spike protein of SARS-CoV-2.

Supplemental Figure 2: Comparison of ELISA data with and without saliva inhibition. Mouse sera containing polyclonal antibodies to Spike protein of SARS-CoV-2 were pooled and

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titrated by ELISA. Saliva from healthy donors were added to serially-diluted antibodies to examine the inhibition rate.

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Table 1: Most abundant proteins absorbed to Spike protein to SARS-CoV-2 as measured by mass spectrometry

Protein	Full name	Ion abundance		
(UNIPROT ID)		Volunteer A	Volunteer B	Volunteer C
MUC7 (Q8TAX7)	Mucin-7	2.72E+07	1.64E+09	1.20E+09
MUC5B (Q9HC84)	Mucin-5B	1.00E+08	1.44E+07	1.83E+07
DMBT1* (Q9UGM3)	Deleted in malignant brain tumors 1 protein	1.45E+09	1.43E+09	7.48E+07
NR1F3/ RORG* (P51449)	Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma)	4.82E+08	1.71E+08	5.48E+07
DEFA1 (P59665)	Neutrophil defensin 1 (Defensin, alpha 1) (HNP-1, HP-1, HP1)	ND	3.42E+08	1.07E+08
DEFA3 (P59666)	Neutrophil defensin 3 (Defensin, alpha 3) (HNP-3, HP-3, HP3)	7.93E+08	3.42E+08	1.07E+08
ZG16B* (Q96DA0)	Zymogen granule protein 16 homolog B	7.88E+07	2.72E+08	1.68E+08
CTSG (P08311)	Cathepsin G	1.06E+08	1.48E+08	1.96E+07
EECP (G8H6I3)	Endocrine and exocrine protein	3.94E+07	ND	8.38E+07
PIGR (P01833)	Polymeric immunoglobulin receptor, PIgR, Poly-Ig receptor	1.01E+08	3.43E+07	3.50E+06
JCHAIN* (P01591)	Immunoglobulin J chain	6.48E+07	4.66E+07	6.10E+06
HIST1H4H/ HIST1H4L/ HIST1H4A (P62805)	Histone H4	6.57E+07	5.58E+07	9.06E+07

ND: not detected

*When multiple isoforms exist, ion abundance from different isoforms were summed.

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Table 2. Maximum inhibition rate of saliva on antibody-binding to Spike protein of SARS-CoV2 and the antibody titer that maximum inhibition was observed.

Donor ID	Inhibition rate	Serum Antibody Titer	Age	Gender
1	52.8%	1:18225	23	M
2	0.0%	ND	24	M
3	7.8%	1:6075	24	M
4	95.2%	1:6075	25	F
5	72.4%	1:6075	26	F
6	16.8%	1:6075	26	F
7	21.4%	1:54675	26	F
8	79.3%	1:6075	29	F
9	60.5%	1:6075	30	F
10	58.2%	1:18225	38	M
11	74.4%	1:6075	39	F
12	84.5%	1:6075	42	M
13	3.8%	1:18225	46	M
14	73.8%	1:18225	50	F
15	13.9%	1:6075	51	F
16	72.5%	1:6075	56	M
17	3.8%	1:6075	60	M
18	83.0%	1:6075	62	M
19	63.7%	1:6075	68	M
20	43.7%	1:18225	70	F
21	0.0%	ND	78	M
22	66.7%	1:6075	82	F

ND: no inhibition was observed at any dilution of antibody

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Zhou et al., Figure 1