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Article

Structure-Based Epitope Design: Toward a Greater Antibody–SARS-CoV-2 RBD Affinity

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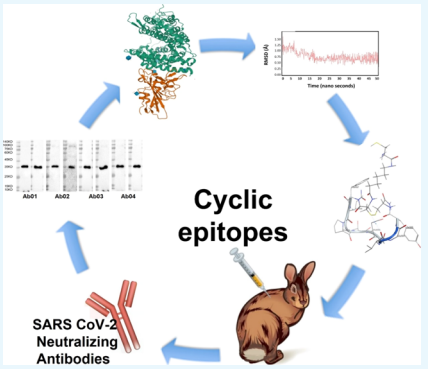
ABSTRACT: Efficient COVID-19 vaccines are widely acknowledged as the best way to end the global pandemic. SARS-CoV-2 receptor-binding domain (RBD) plays fundamental roles related to cell infection. Antibodies could be developed to target RBD and represent a potential approach for the neutralization of the virus. Epitopes used to produce antibodies are generally linear peptides and thus possess multiple confirmations that do not reflect the actual topology of the targeted part in the native protein. On the other hand, macrocyclic epitopes could constitute closer mimics of the native protein topology and, as such, could generate superior antibodies. In this study, we demonstrated the vital effect of the size and the three-dimensional shape of epitopes on the activity of the developed antibodies against the RBD of SARS-CoV-2. The molecular dynamics studies showed the greater stability of the cyclic epitopes compared with the linear counterparts, which was reflected in the affinity of the produced antibodies. The antibodies developed using macrocyclic epitopes showed superiority with respect to binding to RBD compared to antibodies formed from linear peptides. This study constitutes a roadmap for developing superior antibodies that could be used to inhibit the activity of SARS-CoV-2.



INTRODUCTION

Until May first, 2021, more than 150 million people confirmed being infected by the new spreading virus SARS-CoV-2, with more than 2.8 million deaths across the world.[1] Until now, there are no specific efficient drug therapies against COVID-19 caused by SARS-CoV-2.[2] Scientists worldwide are studying preventive strategies and alternatives, including antibodies, vaccines, and antivirals, as well as exploring the potential use of actually proven drugs to treat the infection. In this regard, several promising vaccines are used today to overcome the complications and symptoms associated with COVID-19.[3,4] An antibody-mediated therapy is a promising method to neutralize the proteins of SARS-CoV-2 and thus inhibit its cell penetration.[5−7] Indeed, different proteins are encoding coronaviruses, including S (spike), E (envelope), M (membrane), and N (nucleocapsid).[8] The viral entry into host cells is mediated through the binding between the receptor-binding domain (RBD) of the virus in the S1 subunit and the angiotensin-converting enzyme 2 (ACE2), followed by a fusion between the virus and the cell through the S2 subunit of the protein.[9−11] Thus, the S protein is highly considered as a promising target for discovering efficient antibodies, entry inhibitors, and vaccines.[12] One of the proven methods to prevent and inhibit viral infections is to use antibodies to block the viral S protein's initial binding to the cell's ACE2.[11] On the other hand, antibodies are the most advanced diagnostic tool by far, especially for tests involving fluids such as blood and urine. Antibodies can be applied in the detection of antigens. They can be extended to many technologies such as western blot, ELISA, immunodot blot, flow cytometry and immunohistochemistry, radioimmunoassay, microscopy, and others.[13] *In vitro* diagnostic tests of COVID-19 play an important role in fighting against the current pandemic. Currently, the diagnostic methods used for COVID-19 consist of detecting the virus RNA and antigen besides antibody detection tests.[14] In order to develop fast, specific, and accurate COVID-19 antigen tests, it is important to have in hand high-affinity and highly specific antibodies. Antibodies are commonly used in biomedical fields such as medicine, biotechnology, immunotherapy, and diagnosis, and they are one of the most important biomolecules in life science research.[15−18] Thus, antibodies are considered the fastest-growing drug class where the size of the global market in 2020 is evaluated at USD 130.9 Billion with a growing estimation to USD 223.7 Billion in 2025.[19] However, the production of reliable antibodies remains a challenge and an art and many proteins still lack sensitive and specific antibodies, which greatly restricts or misleads their exploration and validation as drug targets. The sensibility and specificity of antibodies (which directly impacts their performance) are directly linked to the quality of biomedical research

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because antibodies are used in every step of target validation, which is the basis for any drug discovery project. Analyses of target expression, distribution in cells, tissues, and body, as well as how these levels respond to treatment, all rely on antibodies. Antibodies with poor quality, sensibility, or specificity provide misleading results, which jeopardize the few hundred million $ to be invested in this particular biological target.

A critical factor in the production of antibodies of all classes (tools or therapeutic), regardless of the antibody generation platform, is choosing a suitable epitope that determines specificity, selectivity, and sensitivity.[20] The epitope is usually a peptidic sequence which is the moiety physically recognized by the antibody. Once selected and synthesized, the epitope is conjugated to an immunogenic carrier protein to generate a conjugate for subsequent immunization. This is achieved using various platforms and species depending on the final purpose of the antibody (tool, diagnostic, or therapeutic). The epitope is usually synthesized as a linear peptide possessing the same primary sequence found in the protein target. As a result, it possesses multiple conformations that do not reflect its actual secondary structure, that is, its topology, in the target protein. Indeed, protein domains are often information-rich secondary structures such as helices, loops, and sheets, which are ordered topologies not spontaneously adopted by linear peptides. Thus, the use of linear peptides as decoys is limited by the fact that their conformation does not reflect that observed in the actual target. Contrarily to linear peptides, macrocycles are large rings possessing restricted conformations similar to turn structures found in the targeted protein.[21,22] Owing to these conformational restrictions, macrocycles are structurally reminiscent of the native structure of proteins, in contrast with linear peptides. Therefore, macrocycles constitute more suitable conformational epitope mimics for the generation of specific antibodies. Interestingly, the topology of macrocycles can be modulated as a function of the method of macrocyclization, which will be exploited in the current work and differentiates it from other methods. On the other hand, physics-based methods such as molecular dynamics (MD) can predict the biomolecular structures, the conformations of cyclic peptides, and linear peptides with efficient accuracy. The MD simulations process can evaluate the topology and the stability of tested peptides to identify the most stable conformation.[15] A molecular operating environment (MOE) is a computational molecular modeling package that is widely used for a large number of simulations options, such as molecular dynamics, mechanics, and designing and optimizing macrocyclic and linear peptides, proteins, and small organic molecules. This allowed our approach, as opposed to existing competing methods, to thoroughly scan conformational space and ultimately identify a diversity of antibodies that can efficiently recognize the target.

In this work, we have explored theoretically and experimentally the important effect of the size and the cyclization of epitopes on their stability and thus on the affinity of the developed antibodies against the RBD of SARS-CoV-2.

EXPERIMENTAL SECTION

Design of Peptides. Our epitope selection was based on the recently reported crystallographic structure of the complex formed between ACE2 and the RBD of SARS-CoV-2.[23] We have selected one of the regions showing good interactions between the two proteins (Tyr489-Tyr505). The optimized linear and cyclic structures were searched using the low-mode MD application from the "MOE" software MOE 2016.08.[24] All the designed peptides bear a terminal cysteine for bioconjugation with the immunogenic protein BSA.

Molecular Dynamics. The simulations were successfully achieved using the MOE (MOE software).[24] The conformational search was applied in quest of the stable conformation, and the rmsd for each designed peptide was calculated. All hydrogens were added, and energy of the system was minimized. The solvent molecules were deleted before solvation, and salt atoms were introduced to surround the system in a cubic shape. Amber 10:EHT was used as a force field. All electrostatics, van der Waals forces, and restraints were enabled. The heat was increased from 0 to reach 300 K (26.85 °C) gradually, followed by a state of equilibrium. MOE features the ability to perform molecular dynamic simulations for short peptides and proteins. However, the period over which the simulation will be conducted should be divided as it cannot perform for a long duration of time similarly to other programs such as GROMACS. Instead, the MD was started over 10 ns and then the trajectory output was analyzed, and if the equilibrium is not achieved, then the simulation will start for additional 10 ns. The end point here is the steady-state equilibrium of oscillations, and this can be confirmed by the root-mean-square deviation (rmsd) *versus* time plot. Due to peptide flexibility, especially for the long-sequence open-chain peptides, this can need more time. On the other hand, macrocyclic peptides can achieve equilibrium during less time than linear peptides. In this work, the MD was extended to 50 ns to make sure that equilibrium was achieved. The topology was checked before solvation to identify various topological problems present in a residue and can have an effect on the current geometry such as topological restraints; as a result, geometry optimization through energy minimization is performed. In our work, we did not find any topology problems because we have designed short peptides and this step has been performed as a routine step. The system was surrounded by a cube shape of water as a solvent, where NaCl was used as a solute to neutralize the charges in the system. Any deviation was analyzed in the output to measure the average distance between the backbone atoms of the starting conformation and the resulted ones. The start time was zero, and the checkpoint was 250. The molecular dynamics ensemble was Nose−Poincare−Andersen (NPA) to solve the motion equation.

Peptide Synthesis. In this work, we have synthesized linear peptides and macrocyclic counterparts. Syntheses of peptides (**1**), (**2**), and (**3**) were achieved on Rink Amide MBHA resin, while the synthesis of peptide (**4**) was conducted on 2-chlorotrityl chloride (2-CTC) resin using standard solid-phase synthesis with Fmoc chemistry.[25,26] The protected amino acids Fmoc-Cys(trt)-OH, Fmoc-Cys(Boc)-OH, Fmoc-Tyr(tbu)-OH, Fmoc-Pro-OH, Fmoc-Thr(tbu)-OH, Fmoc-Asn(trt)-OH, Fmoc-Gly-OH, Fmoc-Val-OH, Fmoc-Lys-(Boc)-OH, Dde-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Ser-OH, and Fmoc-Gln(trt)-OH have been purchased from ChemImpex International and used directly in the peptide syntheses. A solution of piperidine/DMF (1:1) was applied for Fmoc deprotection. Couplings of amino acids were conducted using a HATU coupling agent during 30 min. For cyclization, two different approaches were applied: for peptide (**3**), the cysteine−cysteine cyclization through the sulfur atoms of the side chains was performed on the solid phase using 0.3 M

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solution of iodine in DMF, while the head-to-tail cyclization for peptide (**4**) was conducted in solution using HATU (Figures 1 and 2). Peptides (**1**), (**2**), and (**3**) were cleaved from the resin, and protecting groups were removed simultaneously using a mixture of TFA/TIPS/H_2O (95:2.5:2.5) for 5 h.

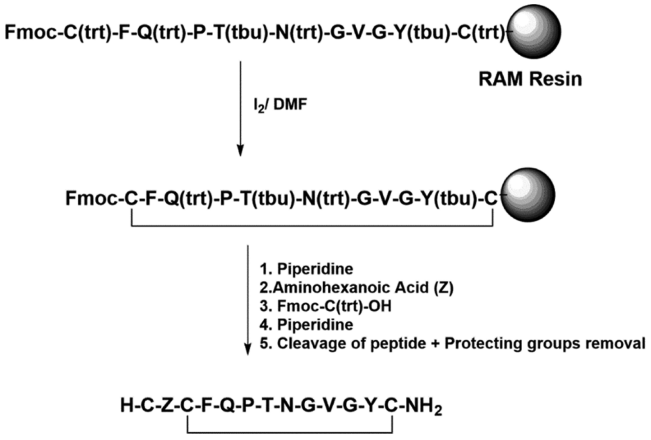

Figure 1. Synthetic scheme for the synthesis of cyclic peptide (**3**).

Before the cyclization of peptide (**4**) in solution, the protected linear sequence was cleaved from the 2-CTC resin using 2% TFA in dichloromethane (Figure 2).

All the peptides were recovered by precipitation in cold diethyl ether and purified by preparative HPLC (Waters Autosampler 2707, UV detector 2489, and fraction collector WFCIII) through an ACE5 C18 column (250 × 21.2 mm, 5 μm spherical particle size) and H_2O + 0.1% TFA and acetonitrile (ACN) as eluents. The purification of peptides was monitored at 220 nm, and the fractions were collected and then lyophilized. Purities of the peptides were determined using HPLC analyses on a CXTH LC3050N system coupled with a UV−visible detector, while molecular weights of the corresponding peptides were verified by LC−MS [Agilent 6125B (ESI) LC/MS].

Immunization and Antibody Production. Immunization and boosts of New Zealand white rabbits for 70 days have been carried out (in duplicate) at Proteogenix (Schiltigheim, France, https://www.proteogenix.science) to produce polyclonal antibodies against the different synthesized epitopes.[27] All the experiments on animals were performed in agreement with the regulations of ISO9001:2015 Quality Management System and the ARRIVE Guidelines (https://arriveguidelines.org). Proteogenix is committed to the ethical use of animal science and their protocols are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Briefly, eight rabbits (randomly male and female) with an average body weight of 2 kg ± 200 g were used. The rabbits were housed in separate metal cages, and fresh and clean water was supplied. Rabbits were kept under appropriate environment and nutritional conditions during the time of the experiments. Immunizations using the different epitopes in conjugation with BSA were performed in a blind and randomized manner. The sera have been collected, and biweekly, ELISA measurements against the corresponding epitopes were performed to detect the antibody production.

Direct ELISA on the Sera against the Corresponding Epitope. Usually, the target antigen is immobilized to a 96-well microliter plate through a chemical reaction that results in the antigen's covalent attachment to the plate via free amino groups. The detection process is then divided into the following three stages, the first of which involves probing the antibody coated well with equal volumes of sera.[28] After that, the bound antibody is probed with a secondary antibody specific for the primary antibody's constant region. The secondary antibody is typically conjugated to alkaline phosphatase, a colorimetric substrate reaction that is used to detect it. After that, the color change is monitored using a plate reader that can detect changes in absorbance after various sera dilutions.

Antigen-Specific Antibody Purification. The antibodies developed in the pooled rabbits' sera were purified using an affinity chromatography technique that involved filtering antibody-containing sera to remove any large interfering particles. The sera were then passed through protein G-coupled Sepharose columns, and the eluted solutions were then passed through agarose beads immobilized with antibody-specific peptides. Then, with a low-salt solution, the unbound nonspecific antibodies were washed out. The targeted antibody is then eluted from the beads and tested for antigen binding using ELISA. After purification, the antibodies were preserved in PBS buffer containing 0.02% sodium azide at pH 7.4.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). The collected fractions were analyzed by SDS-PAGE under reducing and nonreducing conditions at neutral pH. The concentration of polyacrylamide gel was 12%. Samples were incubated with 2% SDS for 10 min at 100 °C.

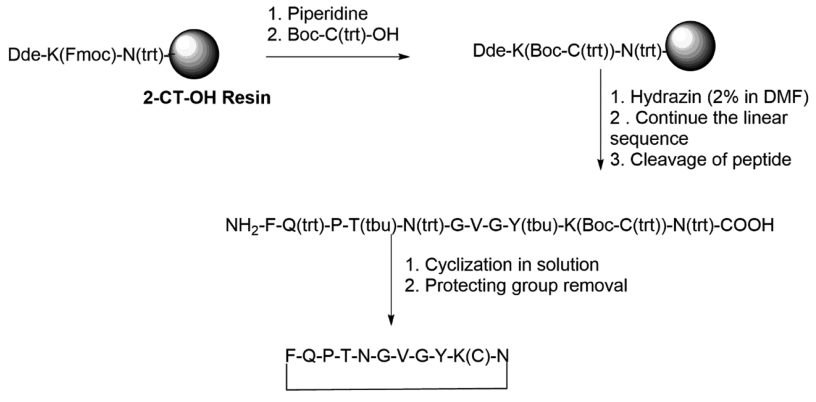

Figure 2. Synthetic scheme for the synthesis of cyclic peptide (**4**).

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Afterward, 200 ng from each sample was loaded onto the polyacrylamide gel wells. The electrophoresis analyses were carried out using the electrophoresis instrument (Bio-Rad Laboratories, USA). After complete separation, the gel was stained with Coomassie Brilliant Blue G 250, and a further distaining step was performed.[29]

Western Blot Analysis of Antibodies against RBD. The recombinant RBD was purchased from Proteogenix. The RBD was electrophoresed on an 8% SDS-PAGE gel and transferred to a nitrocellulose membrane by blotting. The membrane was blocked by bovine serum albumin (BSA) protein. The RBD was detected by adding the purified antibodies and washed, then incubated with secondary antibodies, and visualized by adding horseradish peroxidase HRP-specific substrate, and the color was developed.[30]

RESULTS

Epitopes' Selections and Syntheses. The sequences of the epitopes were selected based on the RBD−ACE2 X-ray structure.[23] All the compounds were synthesized with a purity greater than 90% and verified by LC−MS (SI, Figures S1−S8). The sequences of the four peptides prepared in this work for antibody stimulation are displayed in Table 1.

Table 1. Sequences of the Synthesized Peptides[a]

code	linear peptides	code	macrocyclic peptides
(1)	H-CZYFPLQSYGFQPTNGVGY-NH_2	(3)	H-CZ-[CFQPTNGVGYC]-NH_2
(2)	H-CZFQPTNGVGY-NH_2	(4)	[FQPTNGVGYK(C)N]-NH_2

[a]Z represents the spacer aminohexanoic acid. [] represents the place of cyclization in peptides (3) and (4).

Molecular Dynamics (MD). MD simulations were conducted over a period of 50 ns as an end point at which steady-state equilibrium was achieved for the tested peptides. The trajectories were thoroughly analyzed, and the rmsd (Å) values were calculated (Table 2).

Peptide (3) showed the least rmsd (0.75 Å), achieving continuous stability after 20 ns until the end of MD where the equilibrium was obtained at least deviation (Figure 3). Peptide (3) was top-ranked in the prediction of stability more than cyclic peptide (4) and the linear peptide counterparts that showed higher deviation values more than 1.00 Å (SI, Figure S9).

The macrocyclic peptide (3) and peptide (4) showed the highest energy values (Table 2) where their macrocyclic conformation causes a stress on the bonds and atoms; this was relaxed after MD when the energy computed again to the best conformation at which the stability was achieved. It is worth noting that after obtaining stability, the MD was maintained to ensure that equilibrium was perfectly achieved.

Production of Polyclonal Antibodies. All the synthesized epitopes were conjugated to the protein BSA and used for immunization in rabbits (duplicate rabbits for each epitope). Continuous ELISA against the corresponding epitope was conducted periodically to follow up the evolution of antibody production. After 70 days, all the sera were collected, and the presence of antibodies was confirmed by ELISA (SI, Figure S10). The generated antibodies were purified, and their concentrations were evaluated to be in the range of 1 $mg{\cdot}mL^{-1}$. The pure antibodies were prepared in duplicate and denoted **Ab01**, **Ab02**, **Ab03**, and **Ab04** corresponding to peptides (1), (2), (3), and (4), respectively. The purities of the antibodies were checked using the SDS-PAGE technique under reducing and nonreducing conditions, as displayed in Figure 4, and evaluated to be greater than 95%.

On the other hand, the purity of the RBD that was used for western blot and ELISA studies has been verified by SDS-PAGE under reducing conditions, as shown in Figure 5.

Western Blot and ELISA against SARS-CoV-2 RBD. The recognition between the produced antibodies and the target RBD was initially figured out using the western blot technique (Figure 6).

On the other hand, we have assessed the affinity of our antibodies toward the RBD using ELISA analyses, as shown in Figure 7. The obtained results confirmed the binding observed in western blot.

The EC_{50} or the concentration of antibodies that gives half-maximal binding was evaluated from ELISA analyses for the four studied antibodies **Ab01**, **Ab02**, **Ab03**, and **Ab04**. The obtained values are represented in Figure 8 showing significant variations between the values and indicating an important role of the used epitope in the antibody production against the RBD.

DISCUSSION

In our selected region of RBD (Tyr489-Tyr505), there are eight residues from RBD interacting with ACE2, which could generate antibodies capable of inhibiting the RBD−ACE2 recognition. For further investigation of this region, another subregion (Phe497-Tyr505) has been selected (Figure 9), which allowed us to study the effect of reducing the number of amino acids (from 17 to 9) on the RBD−antibody affinity.

On the other hand, we have prepared macrocyclic epitopes (3) and (4) using the main sequence of the linear peptide (2). These three homologues possess an equal number of amino acids interacting with ACE2 (Table 1). The macrocyclic peptides will give a clear image regarding the effect of the peptide 3D confirmational structure and the way of cyclization on the efficiency of the generated antibodies. The cyclization approach was considered in this work to simultaneously rigidify the peptide backbone, increase its stability, and reduce the entropic cost of peptide−target interactions.[22,31] All the peptides bear a cysteine for bioconjugation with the

Table 2. Computed rmsd Values Resulted from MD Simulations for the Studied Peptides

		calculated energies before MD (kcal/mol)			calculated energies after MD (kcal/mol)		
peptide code	rmsd Å	total E	angle E	Vdw E	total E	angle E	Vdw E
(1)	2.30	470.61	105.73	189.64	392.14	98.84	165.04
(2)	1.69	646.51	91.86	417.83	519.37	81.09	310.47
(3)	0.75	77969.8	281.40	77443.8	5537.22	173.56	54117.45
(4)	1.71	5148.68	121.08	4906.24	4093.07	85.11	3654.71

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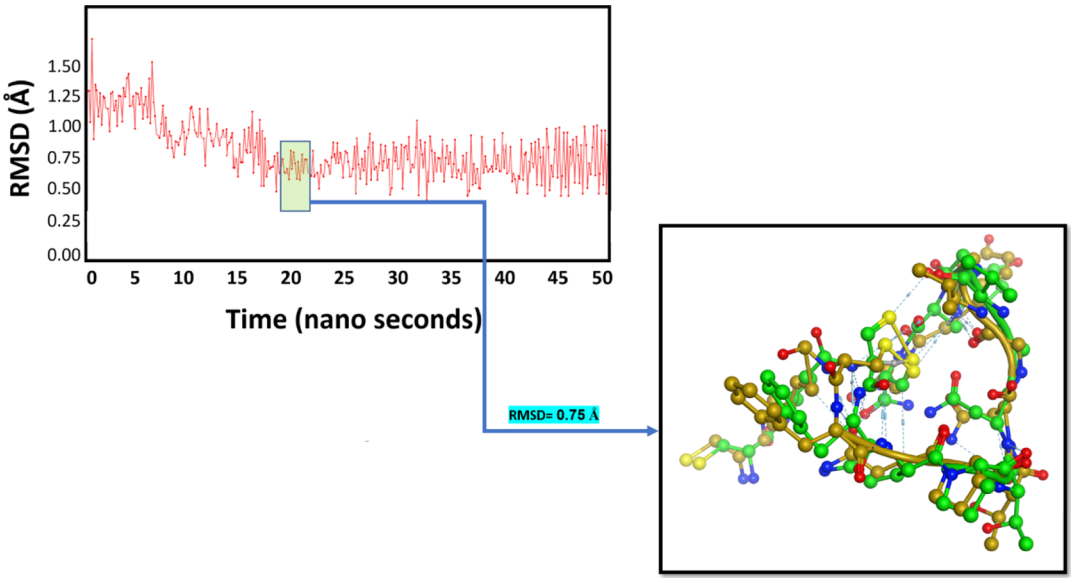

Figure 3. Molecular dynamics of macrocyclic peptide (**3**), showing the area at which equilibrium occurs with rmsd (0.75 Å).

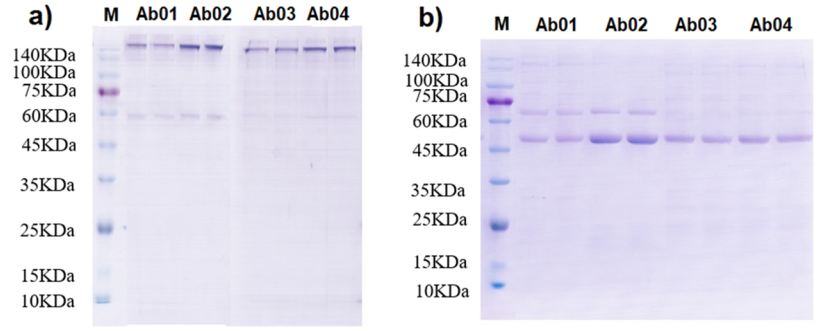

Figure 4. SDS-PAGE for the purified antibodies**Ab01**, **Ab02**, **Ab03**, and **Ab04** under (a) nonreducing and (b) reducing conditions. Each antibody has been produced in duplicate. M is a prestained molecular weight marker.

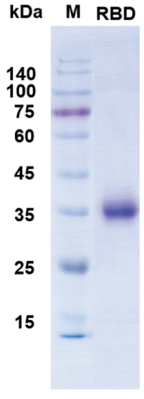

Figure 5. SDS-PAGE for the RBD.

immunogenic carrier protein BSA, which should elicit a strong immune response.[32]

A head-to-tail cyclization mode was also considered in this work. The two cysteine amino acids used in (**3**) were replaced by a lysine and an asparagine in (**4**). Additionally, a cysteine was attached to the side chain of the lysine for the sake of bioconjugation with BSA.

Comparing the MD simulations of the linear peptides (**1**) and (**2**), one can see the effect of the peptide length on their stability (Table 2). Indeed, peptide (**1**) with a longer sequence exhibited a greater rmsd value (2.30 Å) than peptide (**2**) (1.69 Å). This result could be explained by the high flexibility of peptide (**1**) which contains 17 amino acids in the main sequence compared with 9 amino acids for peptide (**2**). Another factor that could affect their flexibility is the type of amino acids existing in the peptide sequence. It has been reported that the most flexible amino acids are glycine and serine, while the most rigid ones are valine, isoleucine, and proline.[33] Thus, the presence of additional glycine and serine in the sequence of peptide (**2**) could contribute to the increase in its flexibility compared to peptide (**1**).

On the other hand, rmsd values of the two macrocyclic peptides (**3**) and (**4**) indicated that the mode of cyclization and thus the 3D conformational structure of the peptide can affect their stabilities. Indeed, compared to a linear sequence, the presence of a constrained cyclization can help in minimizing the unfavorable thermodynamic profile of the peptide.[34] Although macrocyclization of peptides generally improves their stabilities, it has been shown that not all the applied cyclization strategies can improve these properties to the same extent.[35] The smallest rmsd value obtained for peptide (**3**) (Figure 3) demonstrated the important role of the disulfide bond, which could confer additional conformational stability.[36]

The SDS-PAGE technique (Figure 4) under reducing and nonreducing conditions conducted on the produced antibodies

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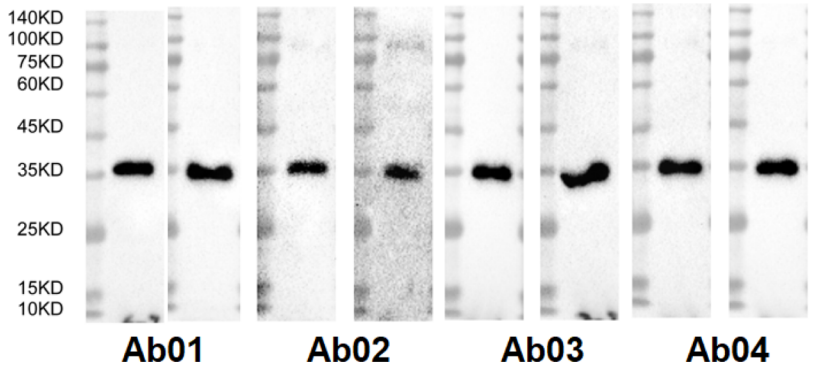

Figure 6. Western blot studies of the produced antibodies **Ab01**, **Ab02**, **Ab03**, and **Ab04** against the RBD. The antibodies were stimulated for each epitope in duplicate (in two rabbits).

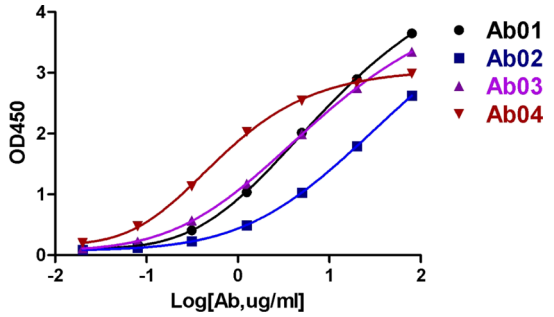

Figure 7. ELISA of the antibodies **Ab01**, **Ab02**, **Ab03**, and **Ab04** against the RBD.

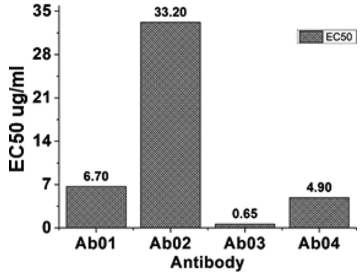

Figure 8. EC_{50} of the produced antibodies against the RBD.

showed the typical molecular weight, which represents the complete antibody entity corresponding to two heavy and two light chains.[37] Moreover, under reducing conditions, the binding between chains was broken down by dithiothreitol DDT, which gives the corresponding heavy- and light-chain molecular weight.[38]

Western blot results (Figure 6) showed a clear recognition between the RBD and the different studied antibodies. This indicates the success of our technique of antibody production using specific regions from the RBD as epitopes. The ELISA data (Figures 7 and 8) demonstrated that the immunized epitopes had generated antibodies with different responses. According to MD simulations, peptide (**3**) was predicted to have better stability as it displayed the least rmsd value (Table 2). This observation was in perfect agreement with our ELISA results, where the corresponding antibody exhibited the lowest EC_{50} and thus the highest affinity toward SARS-CoV-2 RBD (Figure 8). Indeed, compared with its linear counterpart [peptide (**2**)], the macrocyclic epitope (**3**) has generated a more efficient antibody for RBD binding (around 50 times less in EC_{50}). Also, the cyclic peptide (**4**) generated a more efficient antibody compared with its linear control, peptide (**2**), although the MD simulations showed similar rmsd values (Figure 8 and Table 2). These results could be explained by the fact that a macrocyclic, conformationally restricted backbone is a more faithful mimic of the real 3D shape of the surface protein domains than a linear homologue and thus would be able to produce a superior antibody.[22] Additionally, our results showed that the mode of cyclization would generate different antibody efficiencies. Indeed, **Ab03** showed seven times less value in EC_{50} compared to **Ab04**. This result is in agreement with the MD calculations of peptides (**3**) and (**4**) (Table 2) and allows us to suggest that the cyclic peptide through cysteine−cysteine mimics better the native sequence of the RBD. Finally, the effect of amino acid numbers in the epitope on the antibody activity could be seen by comparing the EC_{50} values of **Ab01** and **Ab02**. Indeed, these antibodies correspond to the two linear epitopes (**1**) and (**2**), respectively. Although peptide (**1**) showed less stability in MD due to its highest flexibility, one can see that **Ab01** was around five times more efficient than **Ab02**. This result indicates that the number of amino acids plays an essential role in generating superior antibodies against RBD. Indeed, (**1**) contains 17 amino acids selected from the RBD structure, while the subregion represented by peptide (**2**) has only nine amino acids. Further macrocyclic epitope homologues to the peptide (**1**) should be

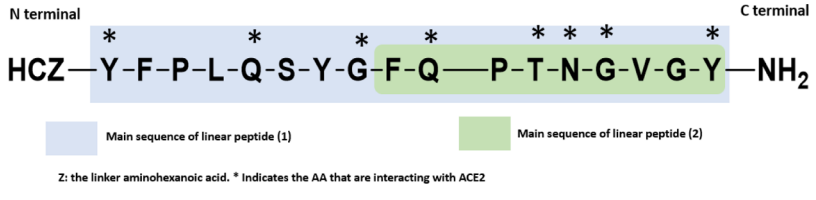

Figure 9. Selected epitope sequences for the two linear peptides (**1**) and (**2**). Both peptides bear an aminohexanoic acid (Z) as a linker and a terminal cysteine (C) for bioconjugation with immunogenic protein BSA.

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investigated in the future to figure out the possibility of improving the antibody−RBD affinity using homologues of peptide (**1**).

CONCLUSIONS

In the present study, we have explored the design of linear and macrocyclic peptides as epitopes for polyclonal antibody production against the RBD of SARS-CoV-2. The design of the examined peptides was conducted based on the reported X-ray structure of RBD in interaction with the receptor ACE2. The design was focused on one of the regions of RBD that showed interactions with ACE2. The designed peptides were subjected to MD simulations over a period of 50 ns, and the rmsd values (Å) were calculated for all the peptides. The rmsd clearly showed an effect of the cyclization on the dynamic stabilities, allowing us to predict the antibodies with the superior activities. Polyclonal antibodies were generated and characterized using western blot and ELISA techniques against the RBD. In agreement with MD calculations, the antibodies that correspond to macrocyclic epitopes showed superior binding affinity toward the RBD compared to antibodies formed from the linear counterpart. Moreover, the type of cyclization and the number of amino acids on the antibody activity were also figured out. Our structure-based epitope strategy should pave the way toward the development of efficient antibodies that could be used in SARS-CoV-2 inhibition.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c03348.

HPLC analyses of peptides; LC−MS analyses of peptides; MD of peptides (**1**), (**2**), and (**4**) showing the reference peptide and the minimized form after MD; and ELISA data conducted on the sera of rabbits immunized with epitopes (**1**), (**2**), (**3**), and (**4**) (PDF)

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Author Contributions

H.T., Y.S.S.A.-F., R.E., and A.N. performed the experimental part; M.A.K. performed molecular dynamics studies; H.T., M.A.K., and A.N. researched data, contributed to discussion and wrote the first draft of the manuscript; H.T., M.A.K., Y.S.S.A.-F., R.E., and A.N. contributed to discussion and wrote and reviewed the final manuscript; and H.T. and A.N. followed the publication process.

Notes

The authors declare no competing financial interest.

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