The SARS-CoV-2 spike protein interaction with an alpaca nanobody

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XXXIX NORDIC CONGRESS IN CLINICAL CHEMISTRY
17-20 SEPTEMBER 2024 STOCKHOLM

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent of an infectious disease called COVID-19. A key, initial step for SARS-CoV-2 to enter the host cells is the specific interaction of the SARS-CoV-2 spike protein with angiotensin-converting enzyme 2 (ACE2) on the cell surface. Despite the development of COVID-19 vaccine, scientists are still paying much attention to strategies to block the binding mechanism between ACE2 and spike protein. Currently, this is the main direction in the development of therapeutic drugs. Antibody therapy is one of the ways to combat the infection caused by COVID-19, but applying of classic antibodies has many limitations. Thus, nanobodies are a good candidate for the treatment of viral infections due to their small size, high stability, good tissue permeability and cost-effective productions [1,2].

The aim of this study is the thermodynamic analysis of the interactions between a selected monomeric alpaca nanobody (named W25) and the receptor-binding domain (RBD) of SARS-CoV-2.

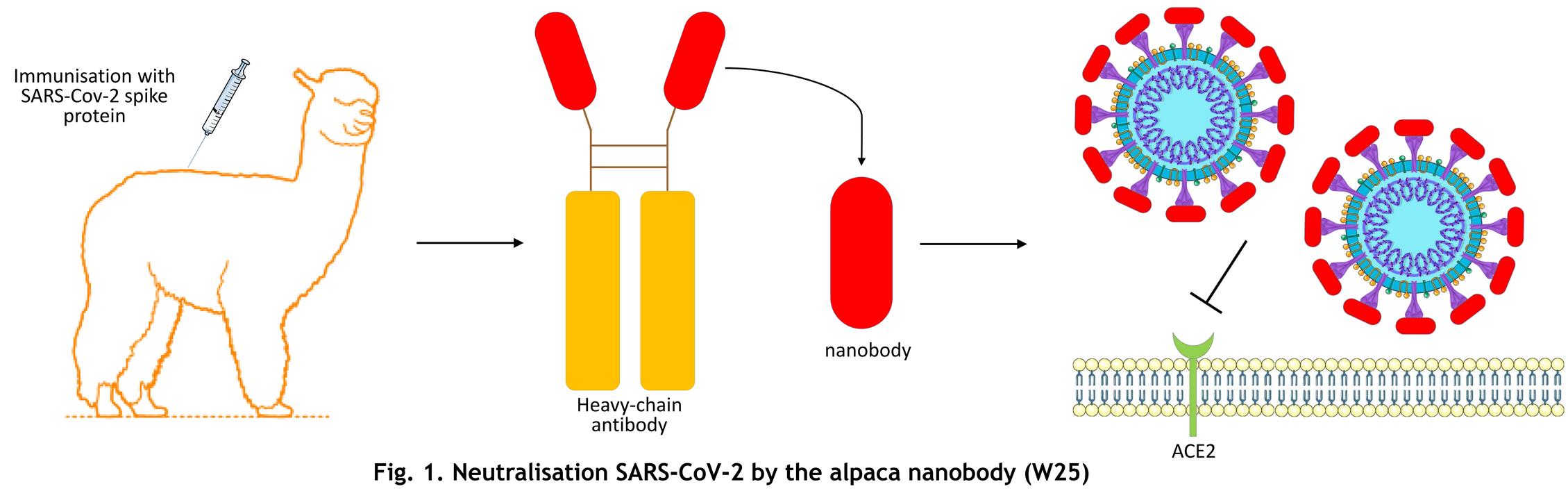

Fig. 1. Neutralisation SARS-CoV-2 by the alpaca nanobody (W25)

Methods

The interaction between the receptor-binding domain of SARS-CoV-2 and an selected alpaca monomeric nanobody named W25 was analysed in detail using isothermal titration calorimetry (ITC). For each ITC assay, the nanobody W25, human ACE2 receptor and RBD were dialyzed against the same buffer (PBS) and during the same time period 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.

Results

RBM of SARS-CoV-2 binds to the hACE2 receptor and the nanobody W25 with a stoichiometry of 1:1 (N_{ITC}), but with very different affinities (K_{DITC}). The stronger affinity of the RBM for the nanobody W25 was found (by ~34-fold) (Table 1).

Both systems are enthalpy driven, but have different ΔG_{ITC} values (the change in free energy)(Fig. 2). The difference can be seen in the ΔS_{ITC}. However, ΔH_{ITC} values are very similar. The enthalpy gain (ΔH_{ITC}) of the RBD and W25 interaction is not sufficiently compensated by an entropy loss (ΔS_{ITC}), resulting in a large difference in affinity (K_{DITC}). When the RBD was pretreated with W25 and subsequently titrated with the ACE2 protein, the binding affinity was significantly weaker (data not shown), demonstrating the effectiveness of the W25 nanobody.

Table 1. Differences in RBD of SARS-CoV-2 binding thermodynamics by the hACE2 receptor and alpaca nanobody W25

	RBD/ACE2	RBD/W25
K_{DITC} [nM]	82.05 ± 20.80	2.37 ± 1.34
N_{ITC}	1.09 ± 0.012	1.02 ± 0.01
ΔH_{ITC} [kcal/mol]	-15.65 ± 0.433	-15.00 ± 0.21
$-T_{\Delta}S_{ITC}$ [kcal/mol]	5.95	3.57

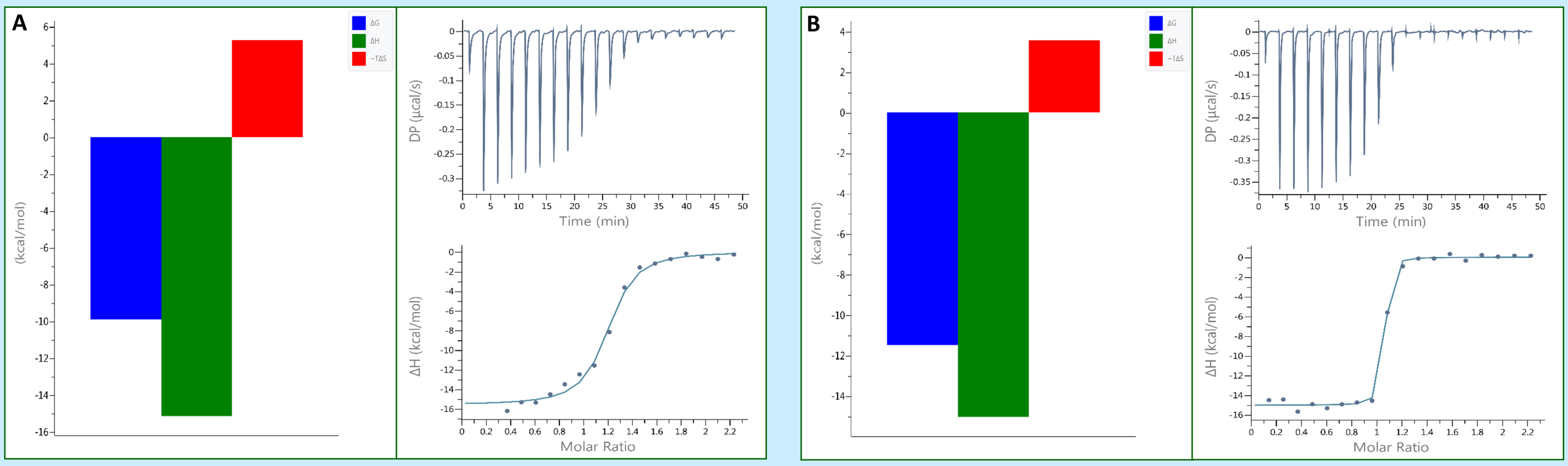

Fig. 2. The calorimetric titration isotherms of binding of RBD of SARS-CoV-2 with ACE2 (A) and with alpaca nanobody W25 (B)

Summary

The results obtained show that the neutralising monomeric nanobody W25 binds to the RBD of SARS-CoV-2 with higher affinity than the interaction between RBD and hACE2 receptor. Our results indicate that the nanobody W25 is a potential antiviral agent.

References

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This work was supported by a grant from the Polish National Science Centre (UMO-2020/37/B/NZ6/01476)

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