ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2022, Vol. 503, pp. 90–92. © Pleiades Publishing, Ltd., 2022.
Russian Text © The Author(s), 2022, published in Doklady Rossiiskoi Akademii Nauk. Nauki o Zhizni, 2022, Vol. 503, pp. 189–191.

BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Among Antibody-Like Molecules, Monobodies, Able to Interact with Nucleocapsid Protein of SARS-CoV Virus, There Are Monobodies with High Affinity to Nucleocapsid Protein of SARS-CoV-2 Virus

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Received October 27, 2021; revised December 12, 2021; accepted December 21, 2021

Abstract—Seven amino acid sequences of antibody mimetics molecules, monobodies, capable of interacting with the nucleocapsid protein of the SARS-CoV virus, were taken from the literature. Nucleotide sequences of monobody genes were obtained by gene synthesis, which were expressed in *E. coli* and isolated using Ni-NTA chromatography. It was shown by thermophoresis that three of the seven selected antibody-like molecules can interact with high affinity (dissociation constant of tens of nM) with the nucleocapsid protein of the SARS-CoV-2 virus. For the remaining four monobodies, only low affinity binding with a dissociation constant of several μM was found.

Keywords: SARS-CoV, SARS-CoV-2, nucleocapsid protein, antibody-like molecules, monobody, thermophoresis

DOI: 10.1134/S1607672922020077

The SARS-CoV-2 coronavirus pandemic has demonstrated the relevance of developing new antiviral drugs. Classical inhibitors of viral activity are low-molecular-weight compounds capable of incorporating into the cavities of viral proteins [1]. However, when viral proteins do not have such cavities, it is difficult to find an effective inhibitor for them. On the contrary, antibodies with high specificity and affinity can be obtained for almost any protein antigen. Moreover, these can be not necessarily natural antibodies but various antibody mimetics, which, in addition to the required specificity and affinity, are much smaller than natural antibodies [2]. For the SARS-CoV-2 virus, the nucleocapsid protein or the N protein can be a potential viral target. It binds to viral RNA and takes an active part in the assembly and packaging of the virus [3, 4], and also has a number of other functions important for the virus, such as replication and transcription of viral RNA [5]. Considering that the homology of N proteins for SARS-CoV-2 and SARS-CoV viruses is more than 90% [6], antibody mimetics that can interact with the N protein of the SARS-CoV virus can theoretically interact with the N protein of the SARS-CoV-2 virus. However, homology does not determine everything here, and in each specific case this assumption can be verified only experimentally. In this study, we used seven monobodies (Fn-N) against the N protein of the SARS-CoV virus, antibody-like molecules created on the basis of the tenth domain of human fibronectin type 3 [7]. The dissociation constants of the Fn-N complex and the N protein of the SARS-CoV-2 virus were determined by thermophoresis. Only three of these seven antibody-like molecules were found to have high affinity for the N protein.

Seven plasmids were obtained by genetic engineering methods, each of which contained a gene encoding an antibody-like molecule with a His tag, as well as a plasmid encoding the SARS-CoV-2 N protein with a His tag (ShineGene). Next, each of these plasmids was transformed with the *E. coli* strain BL21(DE3). Fn–N and N protein expression was induced with 500 μM IPTG for 20 h at 16°C for Fn-N and for 3 h at 37°C for N protein. Fn-N and N protein were isolated from the insoluble fraction [8] and then purified by affinity chromatography on Protino® Ni-TED Resin support. The isolated proteins were stored in a buffer containing 10 mM HEPES and 150 mM NaCl (pH 8).

Denaturing polyacrylamide gel electrophoresis demonstrated a sufficient degree of purity of the obtained proteins (98.9% for the N protein and 92.1, 84.7, 58.1, 81.9, 97.2, 89.1, and 96.7% for Fn-N6, Fn-N10, Fn-N11, Fn-N15, Fn-N17, Fn-N20, and

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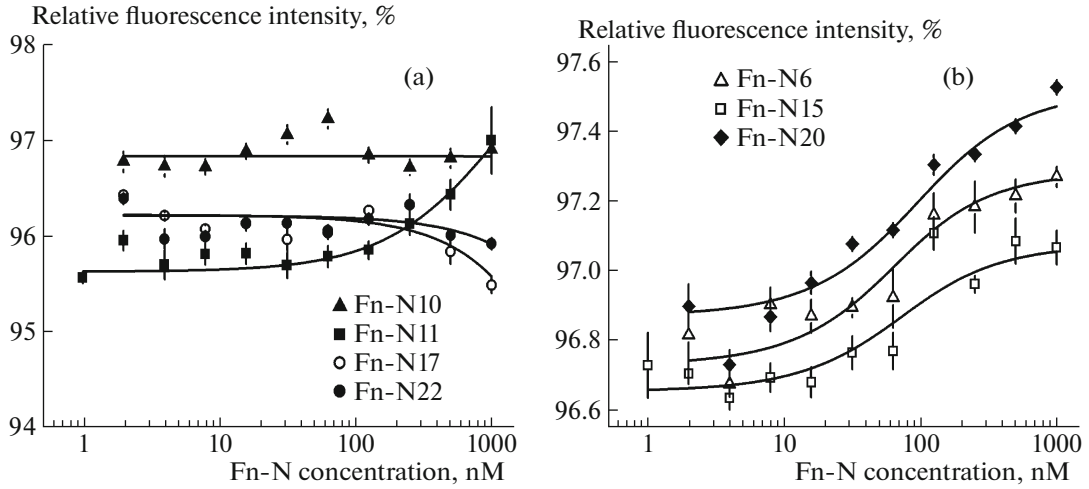

Fig. 1. Dependences of relative fluorescence (fluorescence before the start of thermophoresis was taken as 100%) 20 s after the start of thermophoresis on the concentration of Fn-N at a constant concentration of N protein (40 nM) for Fn-N with low affinity for the N protein (a) and with high affinity for the N protein (b). SEs of relative fluorescence are shown (8–12 replicates).

Fn-N22, respectively). The Fn-N11 protein undergoes partial hydrolysis, which may affect the accuracy of determining the interaction constant of this antibody-like molecule with the N protein.

The interaction of Fn-N with N protein was studied by thermophoresis on a Monolith NT.115 Series device (NanoTemper Technologies GmbH, Germany) in a buffer containing 10 mM HEPES and 150 mM NaCl (pH 7). To prevent aggregation of antibody-like molecules, 70 mM imidazole was added. To exclude the adsorption of the N protein on the capillaries used in thermophoresis, 0.005–0.01% SDS was added to the buffer. The N protein was labeled with the ATTO647 fluorescent dye. To do this, a 1.5-fold molar excess of the activated ester of the ATTO647 dye was added to the N protein in 65 mM carbonate buffer (pH 8.5), and the mixture was incubated for 1 h at room temperature with constant stirring. The N protein with bound ATTO647 was separated from the free dye on a PD10 chromatographic column. Efficiency of N protein labeling was 0.76. The thermophoresis method consists in heating a part of the capillary containing the sample with an infrared laser and measuring the fluorescence of the sample [9]. The level of this fluorescence changes during the formation of a complex between the antibody mimetics and the N protein.

At a fixed concentration of N-ATTO647 (40 nM), thermophoresis was used to obtain dependences of relative fluorescence intensity (fluorescence before the start of thermophoresis was taken as 100%) 20 s after the start of thermophoresis on the Fn-N concentration (Fig. 1). Four such dependences were obtained for each experiment, and the whole experiment was performed in duplicate or triplicate. For each curve, the dissociation constant of the Fn-N complex with the N protein was determined, it was averaged over all 8–12 curves, and the relative measurement error was determined. For Fn-N10, no significant binding to the N protein was observed (Fig. 1a). For Fn-N11, Fn-N17, and Fn-N22 (Fig. 1a), the dissociation constants with the N protein were 2.7 ± 0.6, 3.0 ± 0.5, and 2.9 ± 0.9 μM, respectively. Such high dissociation constants indicate a nonspecific interaction of these monobodies with the N protein. A high affinity interaction with the N protein was observed for Fn-N6, Fn-N15, and Fn-N20 (Fig. 1b). For them, the dissociation constants with the N protein were 31 ± 7, 24 ± 4, and 66 ± 12 nM, respectively.

Thus, of the seven antibody mimetics against the N protein of the SARS-CoV virus, only three interact with high affinity with the N protein of the SARS-CoV-2 virus. Earlier, basing on the high homology of the N protein for these two viruses, Du et al. [10] assumed that Fn-N17 and Fn-N22 would also bind well to the N protein of the SARS-CoV-2 virus. However, our data did not confirm this assumption, and for these antibody mimetics only low-affinity interaction with the N protein of the SARS-CoV-2 virus was observed (Fig. 1a). The Fn-N15 antibody mimetics has the best affinity to the N protein. According to the published data, it interacts with the C-terminal domain of the N protein [7]. Fn-N20, which interacts with the N-terminal domain of the N protein, is of the greatest interest for inhibiting the interaction of the N protein with RNA [7]. Possibly, that is why in the study performed in 2009, of these three antibody-like molecules, Fn-N20 the most effectively inhibited the production of both viral RNA and the viral particles of SARS-CoV [7].

As a result of our study, we have demonstrated that three of the seven previously described monobodies that bind to the nucleocapsid protein of the SARS-CoV virus are also able to interact with the N protein

of the SARS-CoV-2 virus. These antibody mimetics can serve as a basis for the development of antiviral drugs targeted at both SARS-CoV and SARS-CoV-2.

ACKNOWLEDGMENTS

The experiments were performed using the equipment of the Core Facility of the Institute of Gene Biology, Russian Academy of Sciences.

FUNDING

The study was supported by the Russian Science Foundation (project no. 21-14-00130).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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Translated by M. Batrukova