

The Methods and Strategies to Improve the Stability of Nano Antibody

Zimeng Wang

Jinan Foreign Language School, Jinan, China

Abstract: Because of the advantages of simple structure, easy modification, low immunogenicity, good stability, strong specificity and strong affinity, nano-antibodies have broad development space in clinical practice. Compared with traditional antibodies, nano antibodies are easier to store and transport, and after denaturation in high temperature, chemical, pressure and other environments, they can still be efficiently recombined, thereby regaining affinity for antigens. The structure and stability of nano antibody were studied. Multiple DNA sequence repair with the same sequence, easily modified amino acid replacement, protein net charge change, unnatural disulfide bond introduction and CDR supervariable region transfer was used to control the stability of the protein. The implementation of this project is expected to lay a foundation for improving its application in the fields of drug therapy, diagnosis and biosensing.

Keywords: Nano-Antibody; Stability; Structure; Sequence.

1. Introduction

Among them, stability is an important reason to limit the wide use of antibodies. On the one hand, in the production, transportation, storage and use, due to its own instability, it is easy to appear in the production, transportation, storage and use of various physical and chemical properties changes; On the other hand, due to its own unstable aggregation, it may have a greater impact on the yield, shelf life and immunogenicity of the product. Therefore, the stability of the antibody directly affects its biochemical evaluation, but also relates to its purification, storage, formulation design and manufacture [1]. Among them, nano antibodies are chemical denaturation resistance, which are expected to solve the difficult problems such as easy aggregation and decomposition of conventional monoclonal antibodies.

Although nano antibodies are better than conventional antibodies in terms of stability, their stability is very different due to their unique spatial structure. In recent years, many scholars have enhanced their stability by optimizing their common sequences and discovered common structural features that inhibit their aggregation. In this paper, the stability of nano-antibody was studied and its stability in extreme environment such as high temperature and organic solvent was elucidated [2]. The high-charge surfaces, disulfide bonds that restrict conformation migration, more hydrophilic amino acid substitution skeleton regions, conservative hydrophobic regions and strongly interacting functional regions were analyzed [3]. The methods of DNA sequence repair, easy amino acid replacement, protein net charge change, unnatural disulfide bond introduction and functional complementary region (CDRs) modification were discussed.

2. Nanometer Antibody Structure

The novel antibody resembles a conventional antibody variable region (VH) with a skeleton of nine beta-folded

sheets in opposite directions connected by disulphide bonds or interchains (Figure 1 cited in *Biomolecules* 2021, 11(5), 637). The system consists of three CDRs connecting BC, DE and HI terminals respectively, and a continuous surface matching the epitopes is formed through the N-terminal. The skeleton region (FR) between CDRs is a highly conserved domain. Most of the nano-antibody molecules have a double disulfide bond connecting FR1 (C23) and FR3 (C104), and the two betas are connected by covalent bond, thus improving the structural stability of the molecule [4]. Conventional VH and VL are formed by hydrophobic interaction. However, because it contains only three variable regions, it still maintains good biocompatibility with a reduced surface area. This project intends to design and synthesize a series of novel polypeptide molecules with good biocompatibility by introducing a number of hydrophilic amino acids to replace the original aliphatic residues on the binding interface with CH1 and VL. This makes it have high stability, and has a strong stretchable twisted CDR3 structure, so that it is not in direct contact with external water, and can effectively inhibit dimerization. In addition, the hydrophilic core of CDR3 can make it have a larger length of CDR1 and CDR3, so that it has a stronger spatial configuration, which can effectively produce a strong spatial complementarity with the corresponding antigen molecules, and partially compensate for the weakening of binding force caused by the loss of light chain and the sequence variation caused by the small volume.

3. Stability Characterization

In order to achieve the desired effect and shelf life, its physical and chemical properties must be fully studied. In the process of antibody development and application, due to the influence of temperature, solvent, pressure, chemical reagents, acid-base environment and protease, people can evaluate it according to different effects.

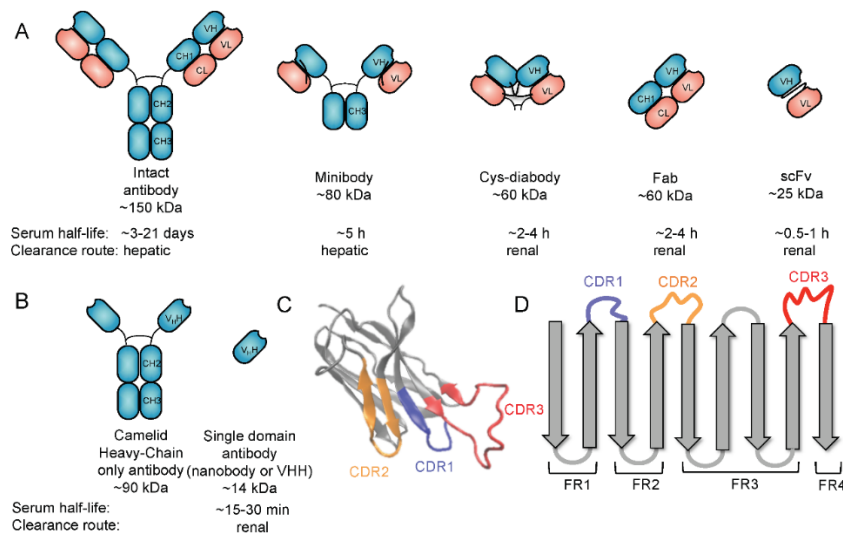


Figure 1. Structure of nanoantibody

3.1. Heat resistance

As an important bioactive substance, it has good thermal stability and good thermal expansion performance. Some nanoantibodies are able to be cultured at -20°C , 4°C or 3°C for several years without losing their ability to bind to the antigen and can therefore be stored, stored and used at room temperature. With the increase of temperature, the folding ability of antibody becomes stronger and stronger, and the folding temperature is usually used to predict the folding trend. The melting point of the new antibacterial material represented by NbA3 can reach 85°C , which is the highest temperature found at present [5]. Due to its good folding ability, the material is expected to be used in high temperature environments (such AS 50°C) for the detection of AS inflammatory indicators. The T_m measurements of conventional Fab and scFv are not much different from those of ordinary nanoantibodies, indicating that there is a certain thermal equilibrium between them, but their reversible properties at ultra-low temperatures are different. After incubation at a certain temperature, the activity of the nanoantibody was measured, and the reversibility of the process could be determined [6]. By summarizing the results of the existing studies, most of the nano-antibodies still have a

certain adsorption capacity after long-term action at temperatures above 85 degrees Celsius, while other traditional antibodies show irreversible inactivation. Anti-caffeine Nb represented by caffeine resistant Nb was incubated at 90°C , and its mAb was easily inactivated at 70°C . Nb, which is resistant to high temperatures, has filed a patent for a device that could be used to monitor caffeine in hot drinks. Previous studies of our research group found that in pasteurization and other environments, the heat resistance of its anti-aflatoxins (Nb-Nb) and anti-unique type antibodies (Aid) was significantly stronger than that of traditional antibodies. After short-term incubation, the binding ability of its polyclonal antibodies (pAb) and monoclonal antibodies only maintained 20% or disappeared completely, and after incubating at the same constant temperature for 20 minutes or even 1 hour, Its specific recognition ability is still 40%-80%, and it is expected to be applied to aflatoxin analysis in complex environments such as pasteurization [7]. Therefore, compared with conventional antibodies, nano-antibodies show greater advantages in complex temperature changing environments. Figure 2 shows the heat resistance experimental process of the nanoantibody (the picture is quoted in Applications of nanobodies in brain diseases).

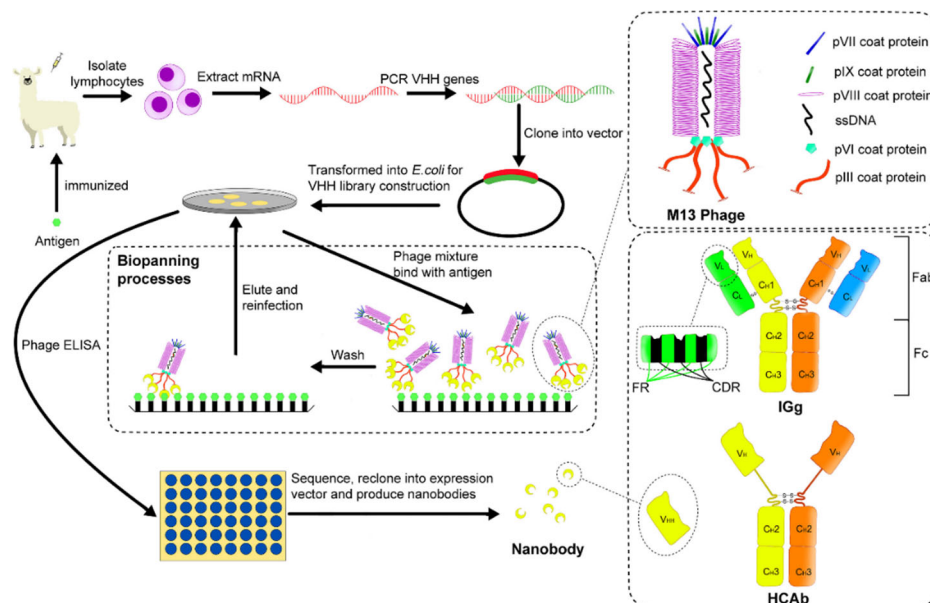


Figure 2. Heat resistance test process of nano antibody

3.2. Organic solvent tolerance

At present, the immunoassay technology used for the detection of lipophilic substances mostly uses organic solvents. However, the existence of organic solvents interferes with the activity of antibodies to a certain extent. Therefore, the interference of these factors must be eliminated by means of volatilization and high proportion dilution [8]. The selection of antibodies with good tolerance to organic solutions can eliminate the volatile resolution process, and can effectively prevent the reduction of detection sensitivity due to high concentration dilution. Studies have shown that most of the nano-antibodies can resist the extraction of high concentrations of methanol, ethanol, acetonitrile, acetone and dimethyl sulfoxide. For example, to separate OTAs from food, methanol must be used, and in this process, methanol not only interferes with the binding of OTAs to antibodies, but also reduces the ability of secondary antibodies. When tested, there was only a small amount of Nb against OTA in the 20% methanol.

This project intends to use the 2.5-fold release method to remove matrix interference, but the detection of monoclonal antibody needs to dilute the concentration of monoclonal antibody more than 30 times, showing a strong ability to resist matrix interference [9]. Taking aflatoxin with strong fat solubility as the research object, niobium has 100% affinity for it at 80% ethanol concentration, but it loses more than 50% of its monoantibody [10]. Its anti-organic solvent property not only can easily determine the lipophilic components in the samples to be tested, but also can be used in the development of low concentration affinity chromatography.

3.3. Stress tolerance

It has been reported that human lysozyme/azo stained RR6 nanoantibodies can denature and fold under high pressure above 400 MPa, revealing the stability mechanism of Nb under high pressure. In addition, the non-covalent protein complex can also be rapidly dissociated under low pressure, so that it can be effectively separated under this high pressure. This method can be used to achieve high selectivity, high selectivity of specific antigens and antigen molecules. At the same time, the technology has the characteristics of high pressure resistance, low dosage and high solubility, which will also lay a foundation for the research and development of a new type of respiratory inhalation drug [11]. At present, NNbALX-0171 trimer is in Phase II clinical trials and can significantly reduce RSV infection in children through aerosol administration. PiN-21, also trimeric Nb, is also stable when sprayed with urea and guanidine hydrochloride and can be used with shampoo to prevent dandruff. However, it is not all negative, for example, Listeria Nb enhances its ability to bind to antigens at high concentrations, suggesting that it may be involved in the refolding of some nanoantibodies.

3.4. pH tolerance

At various pH, the stability of the nano-antibody is basically the same. For example, some scholars have reported that an Nb antibody against tetrabromobisphenol A has good stability in the pH range of 7.4-10. Similarly, Nb of Listeria is not less than 30% active after being cultured overnight in extremely acidic and alkaline environments with pH values of 2 and 12. The new nanomaterials designed in this project will

be affected by the degree of environmental variation detected when used in microfluidic studies.

4. Structural Basis for Stability

Because of its small molecule and single structure, it is more stable than traditional antibodies, but it is not all stable [12]. Previous studies have found that at 65 °C, about two-thirds of people produce irreversible agglomeration, while others remain more than 90% active after heat treatment at 90 °C. Its essence is due to its unique amino acid sequence, the number and location of disulfide bonds and the spatial configuration of its domain.

4.1. Disulfide bond

Disulfide bonds play an important role in the correct folding and stability of cellular efflux proteins. Studies have shown that the expression of nano antibodies using E coli system has significantly lower T_m in the reduced state than in the periplasmic oxidation state, and the difference in their stability is closely related to the formation of disulfide bonds [13]. Under oxidation conditions, the cysteine group underwent double bond oxidation and introduced disulfide bonds into the polypeptide, limiting its non-folded state and enhancing its mechanical properties and conformational stability. Most of these systems have one or more disulfide bonds, one of which connects FR1 (C23) to FR3 (C104) and the other is a disulfide-dioxy disulfide bond that connects CDR3 to other functional regions. The additional disulfide bonds are arranged according to the annular disulfide bond between CDR3 and CDR1 or between CDR2/FR2 and the internal disulfide bond of CDR3 (Figure 3 cited in the General synthetic strategy for regioselective ultrafast) formation of disulfide bonds in peptides and proteins).

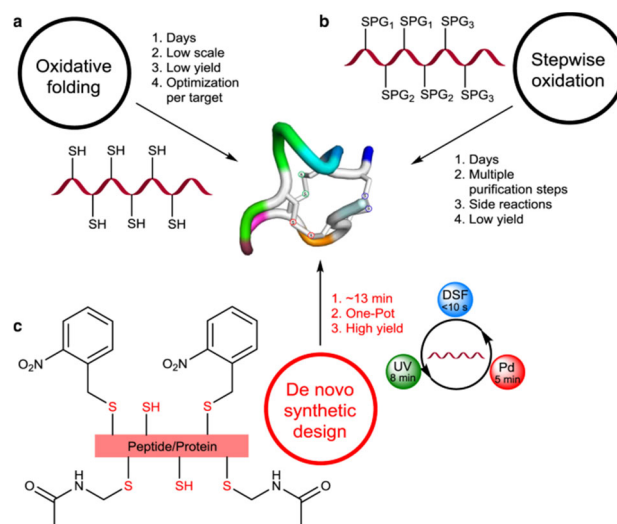


Figure 3. Three different types of additional disulfide bonds in nanoantibodies

The cysteine in CDR1,FR2,CDR2 and other regions is usually located in conserved sites, while the cysteine in CDR3 is randomly distributed, because the cysteine in CDR3 is encoded by the reproductive gene, and the second cysteine site in CDR3 occurs before and after the V-D-J rearrangement [14]. Through the study of their sequencing data, in the nanoantibodies, cysteine residues are usually present in pairs, and this phenomenon is mainly due to its active screening during the development of B cells. The nano antibodies

containing terminal double-chain cysteine are easily oxidized, which makes them lose the binding with antigen, and are prone to dimerization during the preservation process due to long-term preservation. However, there is also a new class of cysteine roots, that is, a new cysteine root, which has a coordination relationship with three other amino acids, and uses zinc instead of disulfide bonds as a bridge connecting the CDR1 and CDR3 ring configurations, enhancing the rigidity of the CDR3 ring configurations.

4.2. Domain

CDRs affect their ability to bind to the target at the molecular level, and their stability under chemical denaturants, temperatures, proteases, and extreme pH depends on amino acid residues. In conventional antibody structure, there are multiple on FR hydrophobic residues (L12, V42 G49, L50, W52, foundry shops, W117), participated in the heavy chain links with CH and light chain. But if it exceeds T_m , this hydrophobic surface will be broken down by conventional antibodies, resulting in intermolecular interactions that cause non-specific aggregation and deposition. In the preparation process, due to its own high solubility, it must be folded with the help of proteins in the self-assembly process. In camels, in order to avoid heavy chain polymerization, some amino acids are mutated, some amino acids are enhanced by hydrophilicity, and some amino acids are surrounded by CDR3, making them insoluble in solution. In particular, on VH, V~ F can fill the hydrophobic region composed of Y95, W117, CDR3 and other side chains, while on W117R, larger side chains of F42 can migrate to Y95 and R117, strengthening the hydrophobic stacking between molecules and improving the stability of the structure. In addition, the V and E residues of FR1 are amino acids common to many structurally stable nanoantibodies, and are also common sites for inducing genetic engineering modification.

5. Conclusion

Although many scholars have obtained nano-antibodies with high stability by site-specific mutation and modification of single sulfur bond, these modifications are based on common sequence and empirical data, and the analysis of their stability is very few, resulting in limitations in stability studies. The synthesis and structure analysis of protein crystals are the most intuitive and accurate means to study protein structure, which can reveal the essence of protein structure from the molecular level. This project intends to construct a spatial structure model of the interaction between a novel nanoantibody and prokaryotes by using methods such as protein crystallization, crystal structure analysis and molecular dynamics, and clarify key factors such as its recognition mode, key amino acid residues, binding sites and force types. The influence mechanism of first-order sequence, second-order folding and third-order spatial structure on its stability was revealed.

References

- [1] Jia Haijing, GAO Yajing, Lou Xinhui. The main research directions, progress and challenges of aptamer screening and affinity evaluation technology. *Advances in Biochemistry and Biophysics*, Vol. 50 (2023) No.9, p.2051-2076.
- [2] Tong Ling, HUANG Guohui, Chen Shanshuang. Anti-gfp nanoantibodies for affinity ligand purification of gap junction protein 26. *Biotechnology*, Vol. 33 (2022) No.3, p.281-286.
- [3] Yan Wenlu, Lang Tianqun, Yin Qi, et al. Progress in improving tumor immunotherapy by actively targeting nanodrug delivery systems. *Acta Pharmacologica Sinica*, Vol. 57 (2022) No.1, p.208-214.
- [4] Wang Yuxuan, Liu Lin, Liu Mingfei, et al. Preparation of GDF15 fusion protein and its application in non-alcoholic steatohepatitis. *Chinese Journal of Hospital Pharmacy*, Vol. 43 (2023) No.3, p.2659-2665.
- [5] Guo Shujing, WANG Xuède, Hu Yuyuan, et al. Advances in lipid based nanoparticles and their use as delivery carriers for phytosterols. *Journal of Henan University of Technology Natural Science Edition*, Vol. 44 (2021) No.6, p.123-129.
- [6] Ren Junwen, Jiang Guoqing, Chen Zhijie, et al. Surface structure design of boron nitride nanotubes and its mechanism of regulating the properties of epoxy composite dielectric. *Acta Physica Sinica*, Vol. 73 (2022) No.2, p.02-08.
- [7] Lv Jingwen. Reliability assignment based on Pythagorean Hesitancy Fuzzy. *Operations Research and Fuzzy Science*, Vol. 13 (2022) No.6, p.106-114.
- [8] Tang Chunxia, Li Meng, Wang Yuxi, et al. Progress in structural design of functionalized cellulose nanomaterials for Cr(VI) removal. *Advances in Chemical Engineering*, Vol. 42 (2022) No.2, p.585-594.
- [9] Liu Yong, Xu Zhewei, Wang Mingwei, et al. Design and properties of porous platinum-based alloy nanoelectrocatalytic materials. *Science and Technology Achievements in China*, Vol. 24 (2023) No.6, p.4-8.
- [10] Kuang H Y, Chen C. Research progress in the design, synthesis and electrocatalytic performance of precious metal nanoframes. *Journal of Chemistry of Chinese Universities*, Vol. 23, 44 (2022) No.1, p.202-209.
- [11] Yang Qhua, Liu Yuheng, Zhang Dandan, et al. Study on respiratory mucosal mRNA vaccine based on polypeptides-ploxamide nanoparticle carrier. *Journal of Army Medical University*, Vol. 45 (2023) No.3, p.1377-1387.
- [12] Bao Wen, Liu Ran, Wang Fei, et al. Basic study of transferrin receptor monoclonal antibody nanoparticle drug delivery system in the treatment of leukemia. *Chinese Journal of Experimental Hematology*, Vol.31 (2022) No.4):939-944.
- [13] Hu Xiangyun, Cao Yan-Hong, LV Lingyan, et al. Nano-antibody and its research status in veterinary field. *Journal of Animal Science and Veterinary Medicine*, Vol.54 (2022) No.8):3164-3172.
- [14] Wu Yue, Sun Baihe, ZHAO Rui, et al. Research progress of therapeutic nanoantibodies in different drug delivery routes. *Chinese Journal of Bioengineering*, Vol.43 (2023) No.1):120-126.