PHYSICS OF MOLECULES

Conference materials
UDC 547.96: 54.03
DOI: https://doi.org/10.18721/JPM.163.263

Determination of the isoelectric point of the antibody to SARS-CoV-2 by molecular modeling for conjugation with quantum dots

S.K. Rudnykh[1]✉, E.D. Gribova[1], K.T. Kholmurodov[1,2],
P.P. Gladyshev[1], N.A. Polotnyanko[1]

[1] Dubna State University, Moscow region, Dubna, Russia;

[2] Joint Institute for Nuclear Research, Moscow region, Dubna, Russia

✉ rudnih@yandex.ru

Abstract. Rapid and effective diagnosis is an integral part of the infectious disease control system. One of the promising directions for achieving this goal is the creation of a biosensor device consisting of a biologically active component (antibody) and a fluorescent label that produces an analytical signal. A necessary condition for the conjugation of proteins with a label is the preservation of their specific activity, since this factor determines the reliability of the analysis result. Violation of the ability of antibodies to form a complex with the antigen directly affects the result of the analysis. A fundamental property of antibodies and other proteins is the isoelectric point, which is defined as the pH at which the macromolecule carries no net electrical charge. Knowing the surface charge distribution and the total pI, it is possible to predict the behavior of the antibody-substrate complex. The isoelectric antibodies to Sars-Cov-2 CA521 FALA (PDB code 7e23) equal to 7.4 were calculated using the molecular modeling method using the Amber complex. Based on the results obtained, the covalent conjugation of this antibody with multilayer chalcogenide quantum dots was carried out by carbodiimide binding in combination with sulfo-N-Hydroxysuccinimide. The quantum dot-antibody complex was tested in an immunochromatographic assay and showed a 200% increase in fluorescence in the test and control zones, indicating successful conjugation under pH conditions below the isoelectric point.

Keywords: antibodies, isoelectric point, molecular modeling, quantum dots.

Citation: Rudnykh S.K., Gribova E.D., Kholmurodov K.T., Gladyshev P.P., Polotnyanko N.A., Determination of the isoelectric point of the antibody to Sars-Cov-2 by molecular modeling for conjugation with quantum dots, St. Petersburg State Polytechnical University Journal. Physics and Mathematics. 16 (3.2) (2023) 360–365. DOI: https://doi.org/10.18721/JPM.163.263

Материалы конференции
УДК 547.96: 54.03
DOI: https://doi.org/10.18721/JPM.163.263

Расчёт изоэлектрической точки антитела к SARS-CoV-2 методом молекулярного моделирования и его применение в конъюгации с квантовыми точками

С.К. Рудных[1]✉, Е.Д. Грибова[1], Х.Т. Холмуродов[1,2],

П.П. Гладышев[1], Н.А. Полотнянко[1]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Университет «Дубна», Дубна, Россия;

[2] Международная межправительственная организация Объединенный институт ядерных исследований, Дубна,

✉ rudnih@yandex.ru

Аннотация. Фундаментальным свойством антител и других белков является изоэлектрическая точка, которая определяется как pH, при котором макромолекула не несет суммарного электрического заряда. Зная распределение поверхностного заряда и общую изоэлектрическую точку белка возможно предсказать поведение комплекса антитело-субстрат. В работе методом молекулярного моделирования рассчитана изоэлектрическая точка антитела к Sars-Cov2- CA521 FALA (код PDB 7e23) и по полученным результатам была проведена конъюгация данного антитела с многослойными халькогенидными квантовыми точками.

Ключевые слова: антитела, изоэлектрическая точка, молекулярное моделирование, квантовые точки

Ссылка при цитировании: Рудных С.К., Грибова Е.Д., Холмуродов Х.Т., Гладышев П.П., Полотнянко Н.А. Расчет изоэлектрической точки антитела к Sars-Cov-2 методом молекулярного моделирования и его применение в конъюгации с квантовыми точками // Научно-технические ведомости СПбГПУ. Физико-математические науки. 2023. Т. 16. № 3.2. С. 360–365. DOI: https://doi.org/10.18721/JPM.163.263

Introduction

Various analytical labels are used to visualize complexes of biomolecules. It should be noted that test systems that use fluorescent labels, which include quantum dots (QDs) are the most sensitive [1]. They have a number of unique properties, including a narrow symmetrical fluorescence peak, high fluorescence brightness, a wide excitation band, and high photostability. Due to these properties, it is possible to reduce the limits of detection and increase the sensitivity of test systems created on their basis. The isoelectric point (pI) is one of the most important characteristics of antibodies (Abs) that affect the interactions of the Abs-QDs system [2]. Currently, there is no physicochemical method for determining the local charge on the AT surface; the influence of various factors on the surface charge can be calculated using molecular modeling [3]. Information about the surface charge of Abs is necessary for the conjugation of antibodies with QDs and obtaining a complex with high analytical characteristics (Fig. 1).

Fig. 1 shows one of the most popular ways of attaching Abs to nanoparticles through amines. Amino groups of Abs are located over their entire surface, and are reactive without any chemical modification with various groups of QDs. In turn, the surface carboxyl groups of QDs must be activated using carbodiimide (EDC) and N-hydroxysuccinimide (NHS) before binding to Abs. The adsorption of Abs on the carboxyactivated surface of QDs is the most acceptable strategy that uses the force of ionic interaction [4]. By controlling the immobilization conditions, the

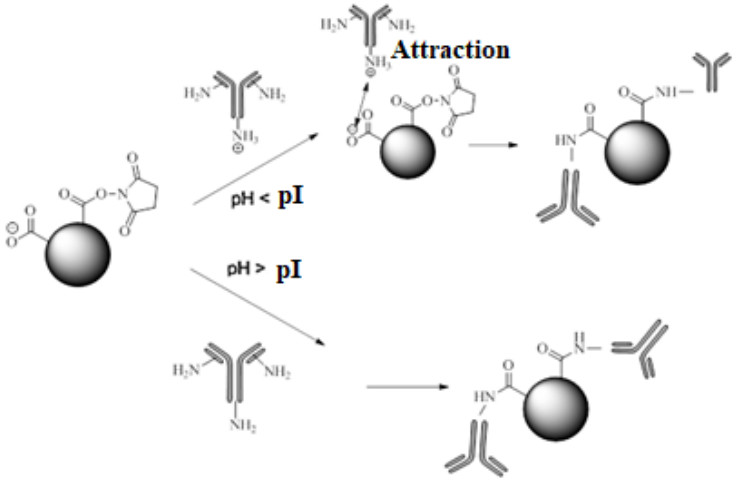

Fig. 1. Carbodiimide binding activating reagents that provide the carboxyl-functionalized QD matrix with a distinct surface charge that can attract AT

carboxyl-activated matrix has fast reversible electrostatic adsorption and covalent binding, and the activating reagent has a significant effect on the orientation of bound biomolecules. As can be seen from Fig. 1, for oriented conjugation, it is necessary to create a pH below the isoelectric point of the protein, since in this case the crystallizing fragment of the antibody acquires a positive charge and, due to electrostatic interaction, is covalently bound by the lower part of the antibody, leaving the antigen-binding pocket open.

Materials and Methods

For molecular dynamics (MD) modeling and calculation of molecular energy minimization within the framework of general mechanics, the Amber complex was used. The electrostatic surface potential of the antibody surface was calculated at different solvent pH. The calculation was performed in PyMol 3.3 (PDB code: 7e23) using the APBS Electrostatics plugin with a range of ±5 kbT/e on the Govorun supercomputer at JINR, Dubna. The calculations are based on the following potentials.

The Lennard–Jones potential models Van der Waals interactions:

$$U(r) = 4E_i\left[\left(\frac{\sigma_i}{r+\delta_i}\right)^{12} - \left(\frac{\sigma_X}{r+\delta_i}\right)^{6}\right], \tag{1}$$

where r is the smallest distance between the side and the surface, E_i is the energy at minimum value, σ_i is the equivalent Van der Waals radius for each residue, δ_i is the dimension parameter.

Electrostatic interaction can be represented through Goui–Chapman potential:

$$U(r) = \frac{\sigma_s q_i e^{-kr}}{kE_r E_0}\ , \tag{2}$$

where q_i is the smallest distance between the side and the surface, σ_S is the surface charge density, k is the reciprocal Debye length calculated from ionic strength.

The net charges of both Ab fragments at different pH values in accordance with the Henderson–Hasselbalch equation:

$$\mathrm{pH} = \mathrm{pKa} + \lg(A^-/[HA]), \tag{3}$$

Results and Discussion

The CA521 antibody (PDB code 7e23) was chosen as a model antibody, since it simultaneously effectively binds all three fragments of the receptor-binding domain of one trimer of the SARS-CoV-2 spike [5]. The structure of this antibody was analyzed according to its charge distribution (Fig. 2,*a*), due to the presence of acidic and alkaline amino acids, as well as carboxyl and amino groups at the ends of both the heavy and light chains, positive and negative charges were unevenly distributed throughout the antibody, which determines the pI of the entire antibody, antigen-binding (Fab)2 and crystallizing (Fb) fragments. The antibody under test consists of 420 amino acid residues formed by 14 amino acids (Fig. 2,*a*). Among which there are acidic amino acids (Arg, His and Lys), basic amino acids (Asp and Glu) and neutral amino acids. The charge of amino acids depends on the pH value of the medium and on the structure of their radical. When the pH decreases, then the H^+ ions present in the solution are attached to the amino and carboxy groups - the charge of the amino acid becomes positive. In the (Fab)2 fragment, the amount of alkaline amino acids was much higher than that of acidic amino acids, while in the Fc fragment they were almost the same (Fig. 2,*b*). According to equation, the total charges of the (Fab)2 and Fc fragments were calculated at different pH values (Fig. 2,*c*). The calculation results showed that the amount of all positively charged residues (basic): 158 (Lys, Arg, etc.) and all negatively charged residues (acidic): 128 (Asp, Glu, etc.) compensate each other at pH 7.4. Thus, the isoelectric point of the CA521 FALA antibody is (pH = 7.4). However, from pH 5.8 to 7.8, the absolute net charge of the Fab fragment was greater than that of the Fc fragment. Even when the net charge of the entire antibody was negative in alkaline environment, the Fab fragment was positively charged and affected the adsorption of the antibody. Thus, electrostatic attraction played a dominant role in the interaction of the positively charged fragment Fab with the negatively charged carboxyl QDs. In contrast, the Fc fragment was less charged, so its hydration shell was thinner and its hydrophobicity stronger. At pH 7.8, both adsorption and covalent

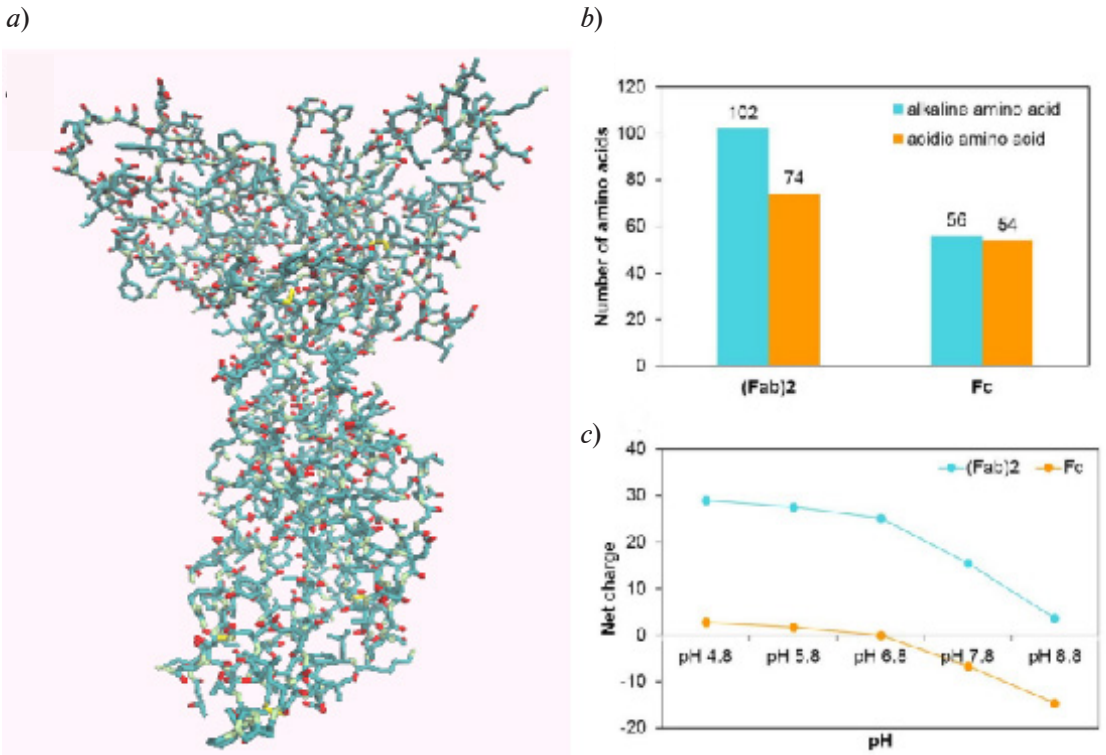

Fig. 2. Results of molecular modeling calculations. Three-dimensional crystal structure of the anti-SARS-CoV-2 antibody CA521 (PDB code 7e23) (*a*). Alkaline and acidic amino acid groups are marked in red and yellow, respectively. The number of alkaline and acidic amino acids on fragments (Fab) 2 and Fc (*b*). Total charge of (Fab)2 and Fc fragments at different pH (*c*).

attachment were unfavorable for tail orientation of the antibody. First, the Fab fragment was positively charged, while the Fc fragment was negatively charged, so that Fab was preferentially adsorbed on the QDs. Secondly, the deprotonation of the primary amino groups of the Fab ends was much more complete than the deprotonation of the side chains of lysine (located in the Fc fragment), and these primary amino groups more easily reacted with carboxyl groups.

At pH 5.8, both the (Fab)2 and Fc fragments were positively charged. In addition, most of the amino groups of the Fab ends were also protonated and thus their cross-linking priority was reduced. In addition, the hydrophobicity of the Fc region was enhanced due to their relatively small net charge, which favors binding to the carboxyl groups of QDs through hydrophobic interaction. Finally, the reduced crosslinking rate gave the adsorbed antibodies time to fully adapt their orientation before forming covalent bonds.

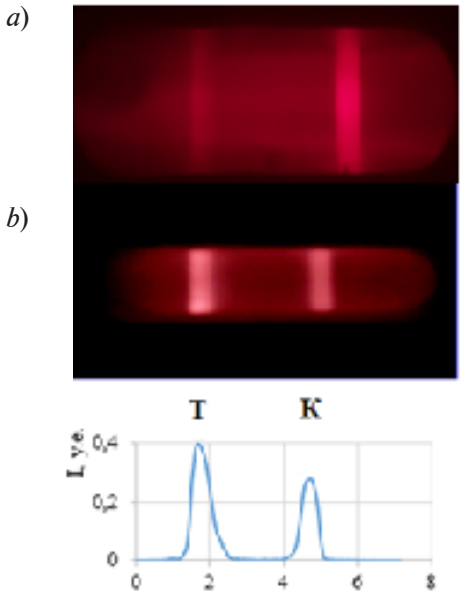

Fig. 3. Images obtained with a fluorescent reader, QDs conjugates at pH 7.8 (*a*), QDs conjugates at pH 5.8 (*b*)

Conclusion

The results obtained were tested in practice, when conjugating AT with multilayer chalcogenide QDs by the carbodiimide binding method. Chalcogenide QDs were obtained by the following procedure [6]. Conjugation was carried out at a value below pI (pH = 5.5) and at a value above pI (pH 7.8), according to the following procedure [7]. The success of the conjugation was assessed by the fluorescence intensity of the AT-CT complex on an immunochromatographic test strip. Fluorescence intensity was measured using a fluorescent reader.

As a result, conjugation at pH 7.8 (Fig. 3,*a*) showed a fluorescence intensity 200% higher than at pH 5.8. The final results can be used in the development of test systems for the identification of pathogenic biological agents with improved analytical characteristics.

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THE AUTHORS

RUDNYKH SERGEY K.
rudnih@yandex.ru

GLADYSHEV PAVEL P.
pglad@yandex.ru

GRIBOVA ELENA D.
elena_g67@mail.ru

POLOTNYANKO Natalya. A.
polot.nat@gmail.com

KHOLMURODOV Kholmirzo. T.
mirzo@jinr.ru

Received 18.07.2023. Approved after reviewing 19.07.2023. Accepted 20.07.2023.