Research Article

Electrostatic Mapping of Rabies Anti-idiotype Antibody Compared to Rabies Virus Glycoprotein

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Abstract.
Rabies is a lethal viral animal disease that assaults the central nervous system. Its glycoprotein is a viral protein that is essential for viral pathogenicity. Initially, the rabies vaccine was produced from nerve tissue, but it is no longer recommended since it causes adverse effects and is less effective. The anti-idiotype antibody vaccination is one option that functions as homologous artificial antigens to the glycoprotein of the rabies virus. The CDR is the structure of anti-idiotypic antibodies that play a role in mimicking epitopes. It may resemble or be identical to epitopes seen in rabies virus glycoproteins. The objective of this study is to determine the affinity of the CDR anti-idiotype antibody for the rabies virus glycoprotein epitope by studying the CDR electrostatic value. This electrostatic value was analyzed by bioinformatics approaches using a webPIPSA server. The findings revealed an electrostatic resemblance between the structure of anti-idiotype antibodies and the rabies virus glycoprotein. Further study will be aimed at collecting electrostatic values from each structure to create an anti-rabies vaccine.

Keywords: anti-idiotype antibodies, CDR, electrostatic, rabies, glycoprotein, webPIPSA

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1. Introduction

Rabies is a contagious viral disease that, when clinical symptoms appear, is almost invariably fatal. This disease is a zoonotic disease, which means that it is spread from animals to people via bites or scratches, most commonly through saliva [1]. Rabies is expected to cause 55,000 deaths worldwide each year, with Asia accounting for 56% of them. In Indonesia, there are nine rabies-free provinces and 24 rabies-endemic provinces [2,3].

Rabies treatment options include post-exposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP) [4,5]. Vaccines can protect against rabies either before or after exposure. Louis Pasteur and Emile Roux created an attenuated rabies vaccine in 1885 [6]. There are four types of vaccines: mouse nerve tissue, human diploid cell cultures, monkey vero cells, and chicken and duck embryos (CCEEVs).[6] Because of their lower immunogenicity, the World Health Organization (WHO) has advised that nerve tissue vaccines be discontinued and replaced with CCEEVs since 1984 [5,6]. Virus attenuation can potentially raise the risk of virulence. Another option is to create an anti-idiotype antibody vaccination [7,8].

Anti-idiotype antibodies act as vaccines by mimicking antigens and stimulating an immune response. TCRs and immunoglobulins have a complementarity-determining region (CDR) with a distinct amino acid structure known as an idiotope. The term idiotype is frequently used to describe a group of numerous idiotopes [9]. Idiotype is frequently connected to an antibody's Complementarity Determining Region (CDR), which specifies specific binding locations. The diversity of antigen-binding sites, notably the CDR hypervariable domain, is connected to idiotypic variability. CDRs are found in both the light and heavy chains, with CDR H3 playing an important role in antigen recognition by changing form upon binding [8,10].

A rabies vaccine based on anti-idiotype antibodies from chicken immunoglobulin (IgY) has been demonstrated to be highly immunogenic and capable of inducing a protective immunological response in animals [11,12]. Animal trials, on the other hand, are time- consuming, costly, and produce superfluous proteins. Recombinant DNA (rDNA) vaccine technology is safer, more effective, and less expensive than traditional approaches [13,14]. The purpose of this work is to compare the epitopes on anti-idiotype antibodies to the rabies virus glycoprotein bioinformatically in order to find an immunogenic epitope for a rabies vaccine based on anti-idiotype antibodies. Protein structure modeling is a technique for determining a protein's three-dimensional structure based on its amino acid sequence. The ultimate goal is to develop a vaccine for the rabies virus that includes anti-idiotype antibody peptides as an immunogenic component to promote protective immunity. Vaccination with anti-idiotype antibodies, which contain the antigen's internal image, has significant advantages over traditional vaccines. Anti-idiotypes are not infectious, but they can evoke a stronger immune response against the pathogen than inactivated viruses and are beneficial for obtaining huge amounts of antigen [15,16].

2. Material and Methods

2.1. Material

The three-dimensional structure of rabies glycoprotein G (PDB ID: 4D6W) and the amino acid sequence of anti-idiotype antibodies (Patent No.: P00201900780) were used in this study [17]. The tools were classified into two categories: hardware and software. A set of personal computers was considered hardware. The software utilized to explore for rabies glycoprotein templates included NCBI BLAST and RCSB PDB. Biovia Discovery Studio 2019 is used to visualize the structure of glycoproteins and anti-idiotype antibodies in three dimensions form. PROCHECK was used to evaluate the quality of the structure that has been formed. The ROSETTA are utilized to build anti-idiotype antibody structures. Then, for electrostatic mapping analysis, use webPIPSA.

2.2. Methods

The study aims to design an anti-idiotype antibody model and conduct a structural comparison between the anti-idiotype antibody and the rabies glycoprotein. The CDR section was then analyzed to determine the structure of the anti-idiotype antibody. The NCBI server was used to search for templates, and a model was created using MODELLER software. The 3D structure was evaluated by PROCHECK.[18,19] The Biovia Discovery Studio 2019 program was used to create a three-dimensional view of the anti-idiotype antibody and rabies G glycoprotein.

The anti-idiotype antibody was marked as a ball on the CDR section and arranged with the glycoprotein's 3D structure in various positions. Compilation results were obtained, and the files were analyzed using webPIPSA to display an epogram diagram illustrating the degree of proximity of structures based on electrostatic values [20,21].

3. Results

3.1. Rabies virus glycoprotein sequences

The protein sequence of the rabies virus is the glycoprotein part. Glycoprotein proteins are antigenic specific components that induce immune system responses. Through the

NCBI GenBank server (http://www.ncbi.nlm.nih.gov/), protein sequences were shown in Figure 1.

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MVPQALLFVPLLVFPLCFGKFPIYTIPDKLGPWSPIDIHHLRCPNNLVVEDEGCTNLSGFSYMELKVGYI
LAIKMNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRYEESLHNPYPDYRW
LRTVKTTKESLVIISPSVADLDPYDRSLHSRVFPSGKCSGVAVSSTYCSTNHDYTIWMPENPRLGMSCDI
FTNSRGKRASKGSETCGFVDERGLYKSLKGACKLKLCGVLGLRLMDGTWVAMQTSNETKWCPPDQLVNLH
DFRSDEIEHLVVEELVRKREECLDVLESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKS
VRTWNEILPSKGCLRVGGRCHPHVNGVFFNGIILGPDGNVLIPEMQSSLLQQHMELLESSVIPLVHPLAD
PSTVFKDGDEAEDFVEVHLPDVHNQVSGVDLGLPNWGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQ
HNLRGTGREVSVTPQSGKIISS WESHKSGGETRL
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Figure 1: The amino acid sequence of rabies glycoprotein.

3.2. Rabies virus glycoprotein model

The NCBI BLAST server was used to identify templates that are comparable to the structure of the rabies virus glycoprotein (Table 1). BLAST is a search tool that searches the GenBank DNA database for sequences that are similar to ours using sequence comparisons.

TABLE 1: BLAST analysis of rabies virus glycoprotein based on the amino acid sequence.

Description	Max Score	Total Score	Query Cover	E-value	Per. Ident	Accession
Chain A, GLYCOPROTEIN G [Chan virus]	66.2	66.2	53%	3e-11	24.24%	4D6W_A
Chain B, C-terminal motif from Glycoprotein [synthetic construct]	30.4	30.4	2%	0.31	100.00%	2KQF_B
Chain A, Spike surface glycoprotein [Human coronavirus OC43]	32.7	32.7	20%	1.7	27.83%	6NZK_A

Table 1 illustrates the results of the BLAST analysis, including the Score, Query Coverage, E-value, and Percent Identity. The score represents the alignment of all database sequence segments that match the protein sequence, with higher scores indicating higher homology levels. The percentage of protein length that is aligned with the database is shown by query coverage. Higher E-values indicate lower homology, while lower E-values indicate higher homology. Percent Identity represents the best match between the query and database sequences being compared.

With a score of 66.2, the chandipura virus glycoprotein has the highest resemblance to the rabies virus glycoprotein, suggesting that 66.2 database sequences match the rabies virus glycoprotein sequences. The Chandipura virus glycoprotein has the highest coverage question, with a 53% E-value. The chandipura virus glycoprotein with PDB ID 4D6W serves as the template for homology modeling, with the structure displayed in Figure 2 corresponding to that in PDB.

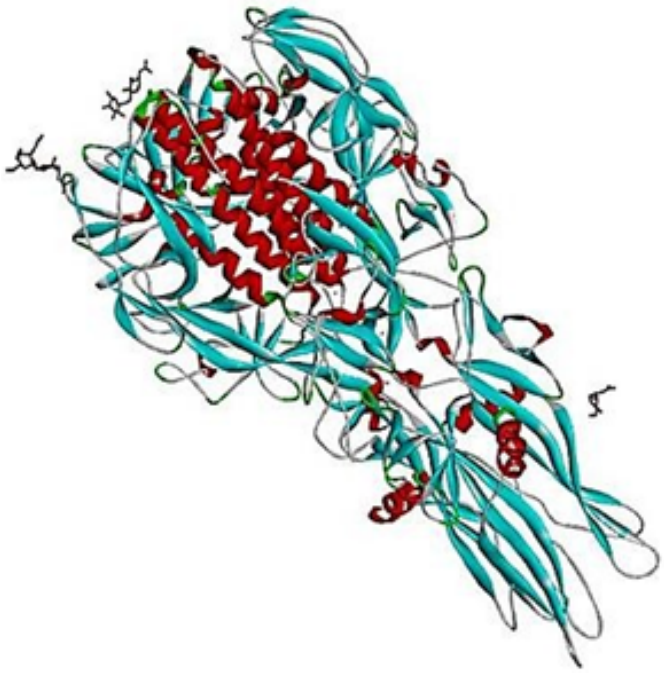

Figure 2: The Chandipura virus glycoprotein structure according to the PDB database.

Figure 2 is the result of validating the structure of the Chandipura virus glycoprotein in the PDB database. The protein structural resolution attained in this experiment was rather low, 3.6Å. resolution is considered to be ideal if it is less than 2Å. The low resolution does not hinder an accurate final model in this case.

3.3. Modeling and evaluation of the rabies virus glycoprotein model

The SWISS MODEL server is used for modeling, and the results are displayed in Figure 3A. The model structure is evaluated by examining overlapping residues and analyzing the Ramachandran plot (Figure 3B). The allowed and generously allowed regions are indicated by light green, while the most favored region is indicated by dark green. The

disallowed region is marked in white. The model is accepted based on data on non-glycine residues in the disallowed region, with a 93.91% percentage of the most favored region.

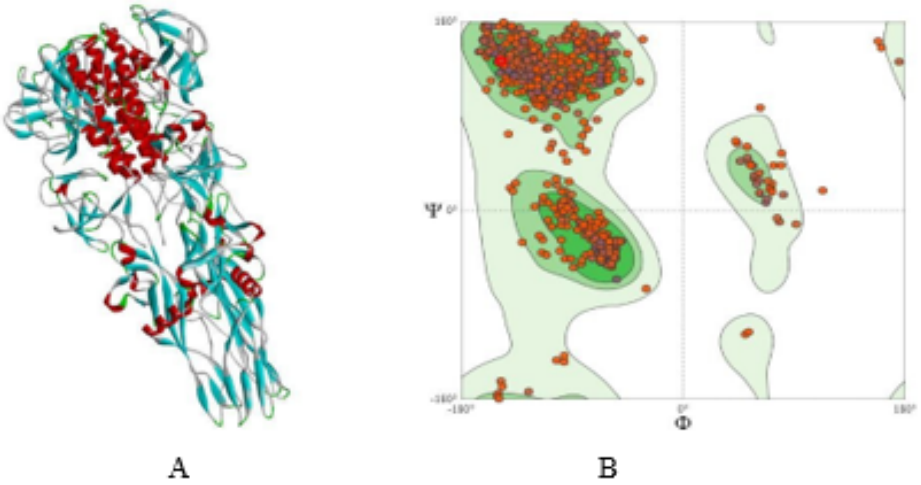

Figure 3: (A) Structure model and (B) Ramachandran plot of Rabies virus glycoprotein modelled by SWISS-MODEL.

3.4. Identification of anti-idiotype antibody templates

The NCBI BLAST server was utilized to explore anti-idiotype antibody templates by evaluating sequences from previous report. 18 As demonstrated in Tables 2 and 3, the results were classified as heavy chains or light chains.

Table 2: Heavy chain analysis of IgY anti-idiotype.

Description	Max Score	Total Score	Query Cover	E-value	Per. Ident	Accession
Chain A, IgY Fcu3-4 [Gallus gallus]	406	449	55%	5e-142	91.23%	2W59_A
Chain A, single chain variable fragment [Gallus gallus]	159	159	24%	3e-45	70.73%	5VF6_A
Chain A, Antibody Scfv 180 [Gallus gallus]	147	147	25%	2e-40	68.94%	4P48_A

Table 2 shows that IgY from Gallus gallus has the highest similarity score with anti- idiotype antibodies, with an E-value of 5e-142. IgY Gallus gallus has the highest coverage and Percent Identity queries, with values of 55% and 91.23%, respectively. The template for homology modeling of the anti-idiotype antibody heavy chain is IgY from Gallus gallus, with PDB ID 2W59_A.

TABLE 3: Light chain analysis of IgY anti-idiotype.

Description	Max Score	Total Score	Query Cover	E-value	Per. Ident	Accession
Chain I, Anti-ptau Light Chain [Gallus gallus]	185	185	90%	6e-59	58.49%	4GLR_I
Chain L, Fab 218 anti-SIRP-alpha antibody Variable Light Chain [Homo sapiens]	201	201	89%	2e-65	58.17%	6NMV_L
Chain A, H5.3 FAB Light Chain [Homo sapiens]	179	179	87%	1e-56	54.81%	4XNM_A

According to Table 3, IgY from Gallus gallus shares 185 similarity scores with anti-idiotype antibodies. It has an E-value of 6e-59, 90% coverage, and 58.49% identity queries. IgY from Gallus gallus, PDB ID 4GLR_I, was used as the template for homology modeling of the anti-idiotype antibody light chain. This is due to the fact that a 3D structure template must be of the same species.

3.5. Modeling and evaluation of the anti-idiotype antibody model

The next stage is to generate the model after determining the template that will be used to create a 3D structural model of the target sequence. The SWISS MODEL server (https://swissmodel.expasy.org) is used for modeling, and the results are shown in Figure 4.

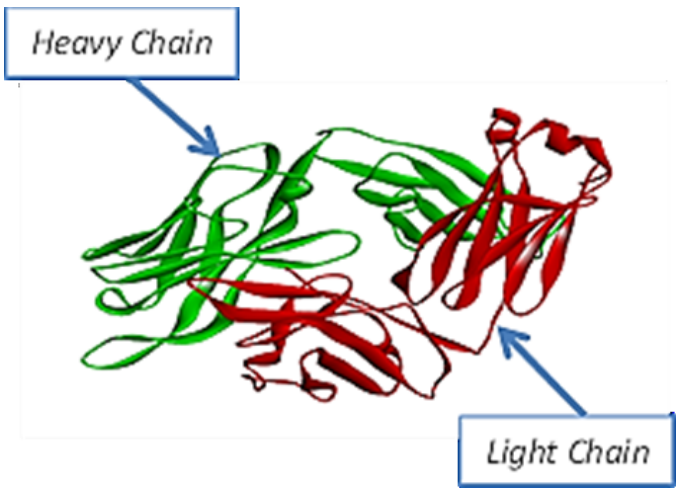

Figure 4: The structure model of the Rabies anti-idiotype antibody.

Figure 4 depicts the 3D modeling of the structure of anti-idiotype antibodies. The structure was divided into two colors: green and red denotes a heavy- and a light-chains, respectively. The model structure is evaluated by examining the GMQE (Global Model Quality Estimation) and Ramachandran plot of the produced model. GMQE is an estimate of structure quality based on target-template alignment. The obtained GMQE value is 0.75, indicating that the model is good. According to Ramachandran's plot, the model is acceptable based on non- glycine residual data. The disallowed region percentage is just 3.3%, while the most favored region percentage is 91.88%.

3.6. The electrostatic mapping value of anti-idiotype antibodies in relation to glycoprotein rabies

Prior to analysis, the CDR part of the anti-idiotype antibody was first determined by changing the appearance of the surface structure of anti-idiotype antibodies with the aromatic, and then given a ball-shaped marker, so that the electrostatic value of the part to be analyzed is only on the structure present within the ball. Aromatic residues, particularly Tyr and Trp residues, are abundant in antigen identification sites and have a significant impact on the process of antibody-antigen interaction. As illustrated in Figure 5, after converting the structure visual to an aromatic appearance, a blue and orange surface is formed, indicating the presence of aromatic residues and most likely the CDR of anti-idiotype antibodies against rabies.

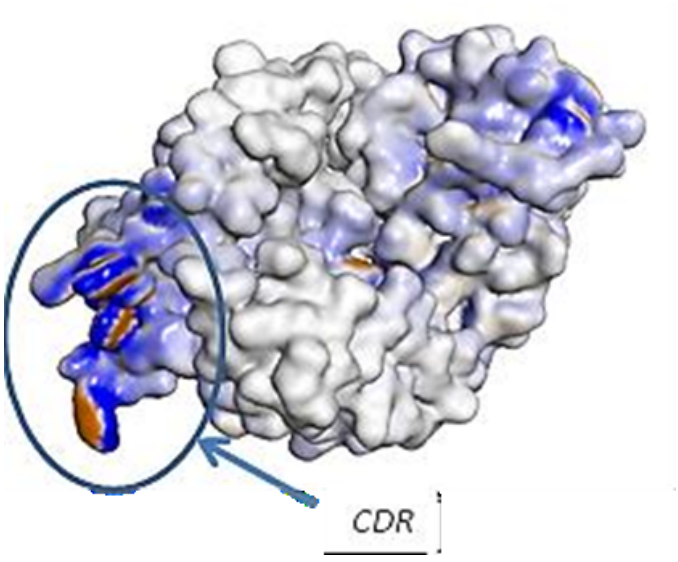

Figure 5: Aromatic residues and potential CDR positions within the structure of antibody anti-idiotype from rabies virus glycoprotein.

The 3D rabies virus glycoprotein structure was docked to a previously generated CDR to analyze the region of interaction (Figure 6A). To detect the entire glycoprotein structure, it was moved to the CDR and compared with anti-idiotype antibodies (assigned as antiabnew). The PDB file format preserves all components imprinted into the CDR, resulting in 27 PDB files obtained from the CDR-glycoprotein structure.

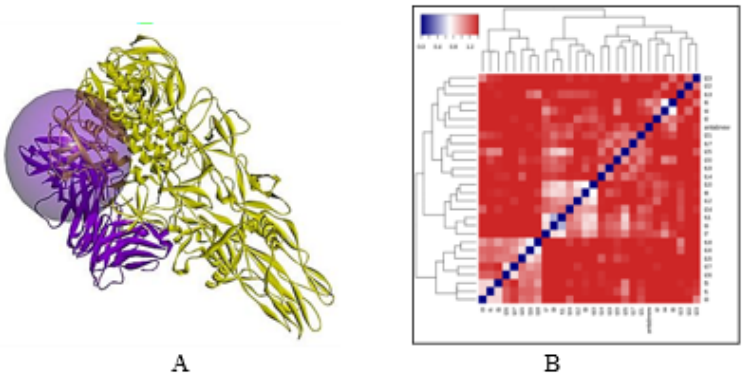

Figure 6: A. Rabies virus glycoprotein structure was docked to a previously generated CDR of anti-idiotype antibodies and B. Electrostatic value heatmap of anti-idiotype antibodies with glycoprotein virus rabies.

The web server PIPSA (https://pipsa.h-its.org/pipsi/) was used to map the electrostatic values of anti-idiotype antibodies with rabies virus glycoprotein. A heatmap of electrostatic values between anti-idiotype antibody structures and rabies virus glycoprotein was demonstrated at Figure 6B. Figure 6B depicts the electrostatic values of rabies virus glycoprotein structures in relation to anti-idiotype rabies antibodies, as indicated by color and values 0-1,4. The blue indicates that the structure's electrostatic values are becoming increasingly comparable to those of other structures, whereas the red indicates that the structure has no electrostatic similarities.

4. Discussion

The relevance of this work is that changes in the electrostatic characteristics of Rabies anti-idiotype antibody compared to rabies virus glycoprotein with their binding properties were identified. Both proteins exhibited a significant number of short-range electrostatic interactions, extremely strong salt bridges, and a very strong salt-bridge at the binding site, as well as the highest electrostatic contributions to binding in, which limit flexibility and making the binding site shape relatively rigid.

Electrostatics have a global and local impact on protein-protein interactions, impacting specificity and affinity by restricting conformational flexibility and modifying structural

and thermodynamic interactions. Because of the lack of substantial electrostatic interactions, the binding site conformation is variable, allowing for greater cross-reactivity with mutant antigens. The overall electrostatic contributions to binding and the scope of the electrostatic environment in protein are determined by the amount and distribution of charged residues [10,16]. The electrostatic characteristics of antibody anti-idiotype and rabies virus glycoprotein differ in this investigation. The enormous number of electrostatic interactions and binding contributions limit flexibility, making the binding site shape stiff. These variances in specificity and affinity can be attributed to these variations [8-10].

Electrostatic interactions affect specificity and affinity directly through complementary polar and charged intermolecular contacts at the binding interface and indirectly through stabilizing partners and their complexes. The quantity and distribution of charges determine the overall electrostatic contributions, whereas the local electrostatic contributions via binding-site salt bridges control the intensities of local interactions [8,9].

Charge mutations affect complexes' association processes, implying methods of association in both complexes. Three main implications may be derived from the findings: changes in electrostatic versus other interactions correlate with differences in antibody specificity and affinity [15]. Electrostatic interactions alter specificity and affinity directly at the binding interface through complementary polar and charged-based intermolecular contacts, and indirectly by stabilizing partners and their complexes, regulating antibody flexibility properties. Electrostatic effects, also known as diffusional electrostatic steering effects, are interactions that occur after a collision that change encounter complexes and transition-state energies [8-10].

5. Conclusion

The study revealed that the 3D rabies virus glycoprotein structure has the most favored region on Ramachandran Plot at 96.6%, with a disallowed region of 1.08%. The 3D structure of anti-idiotype rabies antibodies has the most favored region at 91.88%, with a disallowed region of 3.3%. Electrostatic similarities were found, but electrostatic values were not obtained for each structure.

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