Molecular Dynamics Simulations of a Mouse Monoclonal IgG2A Antibody at the Interface of Polar and Nonpolar Solvent

Institute of Molecular Biophysics

Chase Tarrillo

Dr. Wei Yang, Tianming Qu

Systems and Computational Understanding of Biophysical Actions

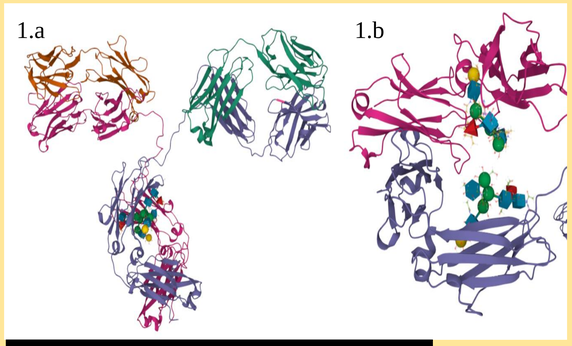
Abstract

Antibodies play an important role in many multicellular eukaryotic immune systems as they are the primary way in which these organisms detect and locate specific pathogens by binding to peptides or surface proteins of pathogens. Due to advancements in expressing and purifying antibodies, it is now possible to treat diseases with antibody therapies [1,2]. It may be helpful to understand the conformation of antibodies in solvents with different polarity, as it can explain extraction equilibria constants and may aid the engineering of future antibodies to be more amicable to extraction or the interaction of a lipid membrane. This paper uses molecular dynamics simulations [3] of a monoclonal mouse IgG2a class antibody in water and in a water-nonpolar solvent interfacial environment [4-6].

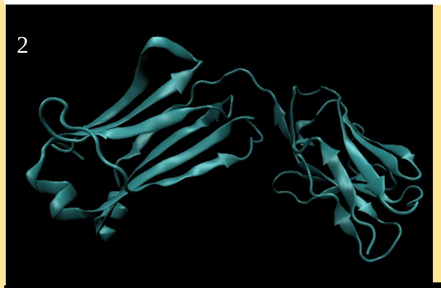
Introduction

Antibodies serve as the intermediary between chemical detection and cellular response in the secondary immune system [2]. Using antibodies, the secondary immune system can recognize the pathogen or allergen based on the antibody that successfully binds to the molecules that accompany an infection, such as the surface proteins or glucans of a bacterial or viral infection, or the toxin itself as in the case for allergens. As antibody structure and effector pathways have been increasingly understood, antibody therapies are being developed to circumvent the lag time it takes for the host to develop effective antibodies [1,2]. The conformational changes of an antibody may be important when designing for efficient purification.

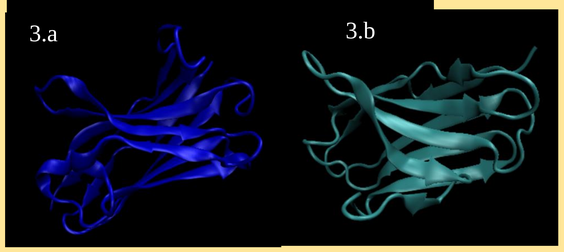
Every antibody subunit's quaternary structure can be described as a dimer of a glycosylated heterodimer [1,2] (Fig. 1a). Each heterodimer has two subunits labeled the Heavy Chain (HC) and the Light Chain (LC), distinguished by their mass and size. Each chain can be further divided into their variable and constant subunits described by their sequence variability, in which the constant regions are highly conserved across classifications, while the variable regions are subject to change to allow for specific binding to antigens. HCs have three conserved regions while LCs have one. The variable regions near the N-terminal of both subunits, of which each chain has one, form the binding epitope of the antibody. The chains of the hetero dimer are then stabilized internally by disulfide bonds and protein surface interaction on the variable regions and the first constant region of each chain. Two intrinsically disordered regions of importance are the elbow region, between the variable and the first constant regions of both chains, and the hinge region, the residues between the first and second constant region of the heavy chain. The heterodimer then combines radially to form a quaternary structure of four subunits through protein surface contacts on the second and third constant HC subunits, disulfide bonds in the hinge region, and the interactions between the carbohydrate chains on the second constant heavy region. The carbohydrate chains are "cradled" by the second and third constant regions of the antibody for the IgG class of antibodies (specific to this poster) (Fig. 1b), which affects the antibody effector pathway [2].



(Fig. 1) a) This is the refined structure of an IgG antibody, with G referring to its class. Chains in orange and teal are Light Chains, while pink and Purple are Heavy Chains. b) This is a closer view of the Carbohydrate Chain attached to the second HC Constant Region. The chains are "cradled" by the latter HC Constant Regions. (PDB entry 1IGT, pictures provided by MOL* from the RCSB PDB [8,12,13])



(Fig. 2) This is a closer view of Chain A from PDB entry 1IGT. The constant and variable regions are observed to be in the characteristic "Immunoglobulin Fold" [2], that consists of 4x3 antiparallel β-sandwich for constant region and a 4x4 for variable regions. The variable regions (the right tertiary unit) contains the Complementary Determining Regions (CDR's) as loops between the β-sheets of the sandwich. These regions create the antibody binding epitope. (Images proceeding are taken using VMD [9])



(Fig. 3) AlphaFold2 predictions for the HV and LV, a & b respectively. They appear similar in structure to other variable regions as seen in fig. 2 as expected. They also appear slightly twisted, like what is seen in fig. 1. The structure was minimized following this step so exact minimums were unnecessary at this stage.

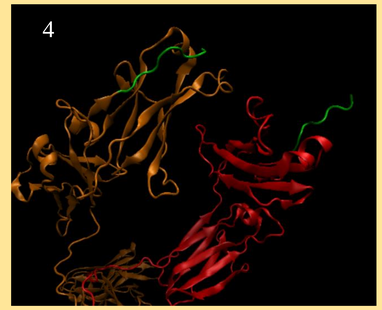
Each conserved or variable region follows the immunoglobulin fold (Fig. 2) [2]. Even within the variable regions, most of the sequence is highly conserved, with most of the variability coming from the Complementary Determining Regions of the variable regions (Fig. 2). Only a few allotypes of each class of antibodies exist, and they only occasionally affect the freedom of movement of the hinge region. This lends one to construct a reasonably equivalent structure using well-characterized structures within the same class as a given antibody

Methodology

Variable Region Prediction: Before MD simulations were performed, a model must have been made. Using AlphaFold2, the variable regions of both HC and LC were predicted. We anticipate this would be successful because of the high conservation between the between antibodies within their own class. It seem AlphaFold2 is well-suited to this task since the program is statistical in nature and it is desirable if the model predicts a structure very similar to structures in the Protein Data Bank [10,11]. The results of the program are in Figure 3.

Template Structures and Refinement: As discussed, high sequence similarity and antibody classification implies high structure similarity. Using the PDB entry 1IGT [8], which has high sequence similarity and is also IgG2a, a model was created by connecting first aligning the variable regions with the variable regions of 1IGT, and then forming bonds in the intrinsically disordered elbow regions of 1IGT. Using Pyrosetta, the remaining residues were then mutated to match the given antibody sequence [14]. While both sequences are IgG2a, they both do not contain four disulfide bonds in the hinge region as one would expect for their class [7,15]. Using Pyrosetta, the TPG tail was added to the C-terminal of the heavy constant region, then refined [14] (fig. 4)

GBSW Membrane Minimization: The structure was then minimized using GBSW Membrane MD simulations to find a reasonably average structure given STP [4-6].



(Fig. 4) The red and orange structures signify different HCs. The green regions signify what was refined using Classic Refine module from PyRosetta [15]. A TPG tail was present in the given antibody, but not in the template PDB (1IGT). The tail was added on the C-terminal end and then refined. The red chain's refined region is most likely non-physical, but it was assumed MD simulations were sufficient to find a region similar to its physical one. It was necessary to have clashing or overlapping atoms to prevent a CHARMM [3] simulation termination.

As of time of writing, the interface simulations have yet to be conducted. *Ask About Results From the Author if Completed by time of Presentation.*

The Author thanks Alissa Anderson at the Nat, High Mag. Field Lab. for her contribution I finding 1IGT and for the detailed description of the antibody.

References

[1] Lu, Ruei-Min, et al. "Development of Therapeutic Antibodies for the Treatment of Diseases." *Journal of Biomedical Science*, vol. 27, no. 1, 2 Jan. 2020, https://doi.org/10.1186/s12929-019-0592-z.
[2] Chiu, Mark L., et al. "Antibody Structure and Function: The Basis for Engineering Therapeutics." *Antibodies*, vol. 8, no. 4, 3 Dec. 2019, p. 55., https://doi.org/10.3390/antib8040055.
[3] Brooks, B. R., et al. "CHARMM: The Biomolecular Simulation Program." *Journal of Computational Chemistry*, vol. 30, no. 10, 14 May 2009, pp. 1545–1614., https://doi.org/10.1002/jcc.21287.
[4] W. Im, M.S. Lee, and C.L. Brooks III "Generalized Born Model with a Simple Smoothing Function." J. Comput. Chem. 24:1691-1702 (2003).
[5] W. Im, M. Feig, and C.L. Brooks III "An Implicit Membrane Generalized Born Theory for the Study of Structure, Stability, and Interactions of Membrane Proteins." Biophys. J. 85:2900-2918 (2003).
[6] W. Im, J. Chen, and C.L. Brooks III "Application of a rotationally invariant procedure to a Generalized Born Model" in preparation (2005).
[7] Vidarsson, Gestur et al. "Igg Subclasses And Allotypes: From Structure To Effector Functions". Frontiers In Immunology, vol 5, 2014. Frontiers Media SA, https://doi.org/10.3389/fimmu.2014.00520.
[8] PDB 1IGT Harris, Lisa J. et al. "Refined Structure Of An Intact Igg2a Monoclonal Antibody,". Biochemistry, vol 36, no. 7, 1997, pp. 1581-1597. American Chemical Society (ACS), https://doi.org/10.1021/bi962514+.
[9] Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Molec. Graphics, 1996, vol. 14, pp. 33-38.
[10] Jumper, J., Evans, R., Pritzel, A. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589 (2021). https://doi.org/10.1038/s41586-021-03819-2
[11] ColabFold - Making protein folding accessible to all Milot Mirdita, Konstantin Schütze, Yoshitaka Moriwaki, Lim Heo, Sergey Ovchinnikov, Martin Steinegger
bioRxiv 2021.08.15.456425; doi: https://doi.org/10.1101/2021.08.15.456425
[12] D. Sehnal, S. Bittrich, M. Deshpande, R. Svobodová, K. Berka, V. Bazgier, S. Velankar, S.K. Burley, J. Koča, A.S. Rose (2021) Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. Nucleic Acids Research. doi: 10.1093/nar/gkab314
[13] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. (2000) The Protein Data Bank Nucleic Acids Research, 28: 235-242. Rcsb.org
[14] S. Chaudhury, S. Lyskov & J. J. Gray, "PyRosetta: a script-based interface for implementing molecular modeling algorithms using Rosetta," Bioinformatics, 26(5), 689-691 (2010).
[15] Dillon, Thomas M et al. "Structural and functional characterization of disulfide isoforms of the human IgG2 subclass." The Journal of biological chemistry vol. 283,23 (2008): 16206-15. doi:10.1074/jbc.M709988200