pK_a Predictions and Electrostatic Potentials of the Anthrax Protective Antigen Protein

Charlene Lee & James M. Briggs[1]

[1]Department of Biology & Biochemistry, University of Houston, Houston, TX 77204-5001

INTRODUCTION

Anthrax is an infectious and sometimes lethal disease caused by the Gram-positive bacterium, *Bacillus anthracis*, which is a spore-forming, rod-shaped bacterium. (Figure 1) The word itself comes from the Greek word for coal (anthrakitis), in reference to the black lesions victims usually develop on their skin. [1] Generally, the disease affects herbivores (cattle, sheep, goats, etc.) that ingest anthrax spores, which are capable of lying dormant in soil for decades. The anthrax eventually kills the animals, and humans that are exposed to dead livestock are often at risk for being infected cutaneously (through skin contact). In recent years, a more deadly form of infection has become better known to the public: inhalation anthrax. This virulent mode of poisoning, caused by breathing in spores, has almost a 100% mortality rate in victims if they are not treated immediately after exposure. [2]

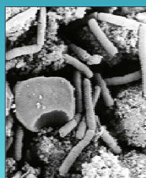


Figure 1: Microscopic view of *Bacillus anthracis*. [7]

The anthrax toxins pose as a large threat to humans, especially through their use in biological warfare; therefore, the research of anthrax and its functions are essential. By studying predicted pK_a values and electrostatic potentials of the central anthrax toxin, protective antigen (PA), a greater understanding the protein's structure and factors that affect its stability can be developed.

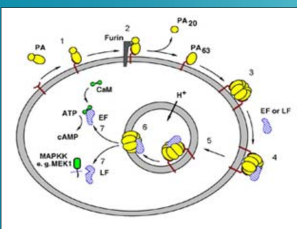


Figure 2: The steps of anthrax intoxication. [8]

After anthrax spores enter the body, they germinate in white blood cells called macrophages and secrete a toxin into the bloodstream. This anthrax toxin is composed of three different proteins—protective antigen (PA), edema factor (EF), and lethal factor (LF)—which are individually benign but work cooperatively to bring about detrimental effects. [3] (*Figure 2*) PA begins as an 83 kilodalton (kDa) protein, called PA_{83}, that binds to host cell receptors and is then cleaved by furin proteases of the host cell into two parts—PA_{20} and PA_{63}. PA_{20} breaks off, and PA_{63} groups itself with other PA_{63} fragments to form heptamer rings, labeled $[PA_{63}]_7$. (*Figure 3*) These heptamer rings act as channels that facilitate the movement of EF and LF proteins into the cytosol of the host cell. [4] Once inside the cell cytosol, EF disrupts homeostasis by increasing concentrations of a messenger molecule (cyclic AMP) that regulates cell functions and thereby causes abnormally high levels of cell fluids and disorder in intracellular signaling. [5] The final protein, LF, is considered the virulence factor of the anthrax toxins. LF cuts off the N-terminus of mitogen-activated protein kinase kinases (MAPKKs), which regulate essential cellular activities like mitosis. More importantly, the cleaving of MAPKKs renders the cell incapable of recruiting immune cells to fight infection.

PROCEDURE

Structural information gathered through X-ray crystallography was downloaded for anthrax protective antigen (PA_{83}; PDB code: #1ACC) from the RCSB Protein Data Bank. (6) In order to successfully run the pK_a prediction calculations, several components of the file (called "cards") needed to be deleted. As a result, only cards displaying information about the individual atoms of the anthrax PA_{83} protein remained. The metal ions of anthrax PA_{83} (calcium ions, Ca^{2+}) were moved and placed right before the last residue of the protein, and any atom cards with multiple atom positions were deleted so that only the first position was listed. A program called "renumber" was then run to renumber all the atom cards in sequential order after removing the unnecessary information.

In order to run the pK_a calculations, an input file containing parameters for the protein was edited. Information regarding the total number of residues, number and location of histidine and cysteine residues, temperature, and other factors needed to be defined. Inspection of the PDB file after renumbering the residues showed that the anthrax protective antigen protein had a total of 667 residues. A Unix command called "grep" was used to search for the number of histidine residues (eight total) and their locations. Within the input file, these histidine residues were all defined to be of type HISA. Had there been any cysteine residues in anthrax PA_{83}, the existence of possible disulphide bonds would have to be located and recorded. The parameters for the remaining specifics—including grid spacing, temperature (293K), and dielectric constants—were left at their default values.

Before actual pKa calculations could be run, polar hydrogen atoms needed to be added to anthrax PA_{83} so that all residues would be in their neutral form. This allows the protein to be in its reference state for the pK_a prediction. The program, called "pkaS-addH" was run, and the output file was checked to confirm that hydrogen atoms were added in reasonable places with reasonable coordinates. Then, another file containing parameters for the anthrax PA_{83} atoms was checked to verify that parameters had been set for the non-amino acid group calcium ions. A final file was edited to define a proper header for the procedure, and the default values for pH and absolute temperature (7.00 and 293.0, respectively) were not changed. After all of the input files were properly adjusted, the pK_a prediction was run using a program called "pkaS-dosbs". Because of the relatively large size of the anthrax PA_{83} protein molecule, some of the programming scripts needed to be altered in order to accommodate it – mainly, the initial grid spacing was increased to 3.0 Å.

Because electrostatic potential calculations were desired, a program called "hyb2uhbd" was run to create an input file for UHBD. The file created was edited by adding defined values and parameters, except the grid spacing had to be changed from 0.80 to 2.0 to accommodate for the large protein size. This file was renamed as an input file and used to run the UHBD program "uhbd_110". The UHBD program created an output file in .apot format containing an electrostatics potential map based on the predicted pKa calculations and the precise net charges for the chosen pH. It was then necessary to convert this file into .phi (Delphi) format using a program called "uhbd2grasp" so that it could be read by the molecular modeling program GRASP. The original PDB file containing structural information about the anthrax PA_{83} protein was read into GRASP, and the electrostatic potential map from the .phi file was overlaid on top of the PA_{83} molecule.

Structures of the heptameric form of PA were also studied and analyzed by downloading its PDB file from the RSCB PDB online (PDB code #: 1TZN).

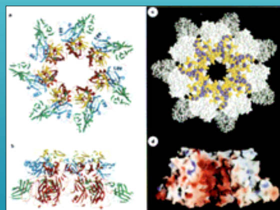


Figure 3: Images of the PA heptamer after cleavage and oligomerization. (a) and (b) represent axial and side views, respectively. (c) shows (a) with a space-filling model, while (d) shows (b) with the surface potentials. The red center indicates a negatively charged lumen. [10]

RESULTS

Atom	Res. Name	Res. Number	Charge
N	SERN	1	0.35
NE2	HISA	73	0.05
CD	GLU	82	-0.97
NE2	HISA	181	0.02
NZ	LYS	195	0.95
NE2	HISA	278	0.09
NZ	LYS	448	0.81
NE2	HISA	527	0.52
CD	GLU	575	-0.96
CG	ASP	578	-0.97
CD	GLU	580	-0.98
CD	GLU	584	-0.96
C	GLYC	667	-1.00

Figure 4: Table of shifted residue charges at pH 7.

The residues to the left have fractional charges that vary more than 2% from the expected charges at pH 7.

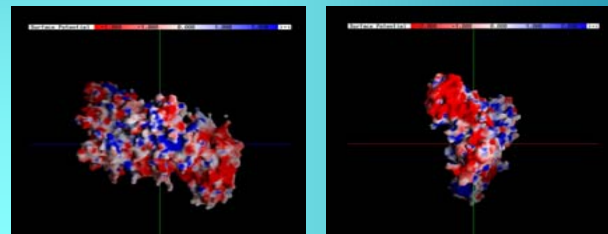
Aspartic acid (ASP) and Glutamic acid (GLU) should all have had values near -1.00, while Lysine (LYS) and Argrinine (ARG) were expected to have charges near +1.00. Histidine (HIS) residues should have had net charges between 0.00 and 1.00.

These charge variations are most likely caused by the ionizable residues' interactions with adjacent or nearby charged and polar residues.

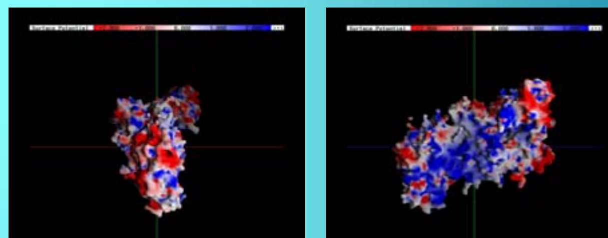
HIS Res. Number	Charge
73	0.05
181	0.02
223	0.00
233	0.00
257	0.00
278	0.09
527	0.52
546	0.00

Figure 5: Table of histidine residue charges at pH 7. The ionization states of histidine residues tend to vary because its pK_a value is near 7.

Figure 6: Anthrax PA_{83} model displayed with secondary structures using VMD. [12]



Electrostatics potential maps of the anthrax protective antigen are visualized using GRASP. [7] Each image displays a 90 degree clockwise rotation of the protein about the y-axis (starting from the top left hand corner). Red represents negative surface charges, white indicates neutrality, while blue represents positive charges.



CONCLUSION

- •The calculated ionization states of most residues of are agree with the predicted values.
- •The electrostatics maps show that PA_{83} has a negative (red) side, confirming the presence of a negatively charged lumen after oligomerization. The existence of a negative central pore is consistent with the idea that EF and LF proteins are predominantly positive and therefore transport easily into the cell.
- •Further studies of blocking antibodies need to be conducted in order to determine ways to prevent the PA_{83} heptamer from transporting the other two anthrax toxins, EF and LF, into the host cell.

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