Quantitative Analysis of HBV Capsid Protein Geometry Based Upon Computational Nanotechnology

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ABSTRACT

Computational biology is an interdisciplinary field that applies the techniques of computer science, functional mathematics and statistics to address problems inspired by biology. Numerous studies have shown that structure of proteins contribute towards their functionality in a direct or indirect way. The structure of viral capsid proteins are critical for hosting and shielding the genetic material and are also crucial for viral entry. In this work, nano-environmental energetics of mutated Hepatitis B capsid protein (HBc) dimer was studied for determining the stability of the protein. Results provide structural information regarding the important residues contributing to Hepatitis B virion (HBV) synthesis. This work illustrates the salience of computational nanotechnology and paves the way for future pharmaceutical applications aimed at destabilization of the capsid-surface protein interactions.

Keywords: Nano-environmental Energetics, Point Mutation, Bioinformatics, Hepatitis B virus, Structure function relationship

1 INTRODUCTION

A variety of computational tools exists for molecular and structural analysis that can perform amino acid mutations, calculate H-bonds and distances between atoms quite easily [2]. Many bioinformatics tools are available to analyze structures in molecular level, to study the different interactions in nano-scale and determine the energy in this nano-environment. The different interactions that exist at this level are charge-charge interaction, hydrophilic-hydrophobic interaction, steric effects and bond stability [5]. Structure function relationship of proteins has been studied and structure has been shown to be closely related to the functionality of the protein [6]. In virions, the capsid proteins are nano-scale structural proteins that protect the nucleic acids of the virus. Hepatitis B virus (HBV) is one of the smallest enveloped animal viruses with a virion diameter of 42nm [1]. The major building block of the HBV is the core protein (HBc) [7]. The core protein contains 183 amino acids. Previous work has been done to record the dimensions of the HBc experimentally [3] [4]. Two bundles, each of length 4.2 nm, run from the inside of the shell to the extremity of the spike. The two bundles are joined at the tip of the spike by a loop. They are the anti-parallel alpha helical hairpins. Each dimer is made of four such helical hairpins which have formed the dimensions of the spike [7]. The total length of the alpha helical spike region is 12.2 nm which corresponds to 81 amino acids. The dimensions of the five alpha helical domains are taken as the five parameters of the HBc protein dimers. Parameter A contains amino acid 27 to 35 and of length ~1.5 nm, parameter B contains amino acid 48 to 78 and of length ~4.2 nm, parameter C contains amino acid 82 to 94 and of length ~4.2 nm, parameters D + E contains amino acid 94 to 110 and amino acid 112 to 128 of total length ~2.3 nm. The tips of the dimer were in contact with the envelope via protrusions that emerged from the inner surface of the envelope [9].

2 METHODS AND METHODOLOGY

2.1 Choice of Model Parameters

All measurements and nano-environmental energetics measurements were carried out in The Swiss-PdbViewer [11], a bioinformatics tool used for computational analysis. The dimension of the alpha helical regions of an individual dimer that were obtained using bioinformatics method are used for comparison between the point mutated HBc protein dimer and the HBc protein dimer present on the wild-type hepatitis B virion. Parameters obtained bioinformatically are given in table1. The HBc protein consists of four chains namely A, B, C and D. Chain C is taken for the comparative study.

2.2 Quantitative Nanoscale Energetics

Initially the five parameters defined above were measured and recorded for the wild-type HBc protein dimer for chain C. Then the point mutations, as given in table 2 were induced in chain C, in their appropriate position. The mutated dimer in this stage is in its most stable form. After this, hydrogen bonds are computed for the dimers. Next molecular surface is computed. Electrostatic potential is then computed. Coulomb's method is used for the computation of the electrostatic potential. Force field energy is then computed and the result contains the total energy with all the energy contributing factors. The summation of all these energy values, provide the total

energy of the selected dimers. Nano-environmental energy minimization is then carried out for the entire structure containing the four dimers. Nano-environmental energy minimization is applied to this problem so as to obtain the changed structure after point mutation, because this energy minimization can repair distorted geometries by moving atoms to release internal constraints in nanoscale structures. Moreover, this energy minimization is usually performed by gradient optimization i.e atoms are moved so as to reduce the net forces on them. The minimized structure has small forces on each atom and therefore serves as an excellent starting point for molecular dynamics simulations. After this the final nanoscale structure is obtained and the five nanoscale parameters are measured and compared with the parameters obtained for wild-type hepatitis B virion.

2.3 Domain selection and Point Mutation

The amino acids selected to perform point mutation on the capsid dimer on chain C are listed in table 2. As it can be clearly seen from table 2, the amino acids selected to perform the point mutations are Arginine, Glutamine and Asparagine. This is because, from the force field energy calculation, these amino acids were found to contribute maximum towards the stability of the virion. Moreover, it is always ensured that the replacing amino acid causes maximum structural change for the HBc protein dimer, which is decided based on ΔG_a [8] value. A negative value of ΔG_a is selected because it is destabilizes the HBc protein.

3 RESULTS

Comparing the value obtained in table 1 for chain C with the parameters obtained after point mutation in table 3, the following observations were made. The maximum change in the five parameters combined together (A+B+C+D+E) is obtained for the point mutation Q99H (12.4113nm) followed by R82K (12.3244 nm) and then N75W (12.3137 nm). The difference in value of the five parameters combined together for chain C between mutated and wild-type HBc dimer, varies between 0.01544 nm to 0.02675nm. These five parameters represent the key geometric factor determining the dimension of the dimeric spike present on the nucleocapsid of the HBV. Even though this difference is in the order of 0.01 to 0.03 nm it has significant effect on the structural and functional effects of the HBV virion. In wild type HBV virion, it has been found that, the tip of the spikes contact the envelope via protrusions, emerging from the inside surface of the HBV envelope. This clearly indicated that contact is formed between the core dimeric spikes and HBs protein [9]. Image reconstruction of the various gapped and compact HBV has identified the tip of the spike as the major interface between HBc protein and the HBs protein. It has been found out that structural changes at the tip of the spike may alter the charge distribution in such a way that the affinity of the nucleocapsid to the envelop enables control of envelopment [9]. In the point mutation analysis in this paper, the dimensions of the spike vary in accordance with the various point mutations made, which in turn ensures that the position of the tip of the spike changes and hence it will affect the functionality of the hepatitis B virion. It has been found by experimental methods that point mutation in ASP78 abolished nucleocapsid formation completely [10]. Moreover, it has been shown that ASP78 is a potential site of contact between the HBc protein dimeric spike and HBs protein [9]. In our bioinformatics study, point mutation is performed in ASP78 with Tryptophan to see the change in the five parameters (table 5). Tryptophan was selected to perform the mutation since it had negative value of ΔG_a (-2.15 kcal/mol) and hence destabilizing. Any other amino acid having negative value of ΔG_a could have been selected but Tryptophan was found to produce the maximum structural change.

Table 1. Parameters of Chain C in wild-type hepatitis B virion obtained bioinformatically.

S.No	Parameter	Amino Acid	Chain	Length
1	A	27 - 35	C	1.2050nm
2	B	48 - 78	C	4.4745nm
3	C	82 - 94	C	1.7443nm
4	D	94 - 110	C	2.2969nm
5	E	112 - 128	C	2.4231nm

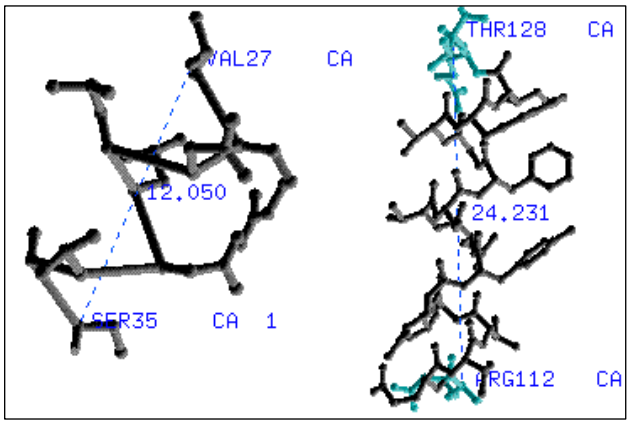

Figure 1: Screenshot providing support to data presented in table 2, parameter A (left) and parameter E (right).

Table 2. Amino acid selected to perform point mutation in Chain C.

S.No	Chain	Residue ID	Amino Acid present in wild-type HBV dimer	Amino acid with which point mutation is performed
1	C	39	Arginine	Valine
2	C	57	Glutamine	Glycine
3	C	75	Asparagine	Tryptophan
4	C	82	Arginine	Lysine
5	C	99	Glutamine	Histidine
6	C	133	Arginine	Cysteine

Table 3. Results of nanoscale energetics measurements based upon point mutation analysis on chain C.

S. N0	Chain	Residue ID	Amino Acid present in wild-type HBV dimer	Amino acid with which point mutation is performed	Accessibility of solvent (in %)	Torsion angle (φ,ψ)	Parameter A (in nm)	Parameter B (in nm)	Parameter C (in nm)	Parameter D (in nm)	Parameter E (in nm)	Predicted ΔΔG value (kcal/mol)
1	C	39	Arginine	Valine	33.19	-34.1°, -74.9°	1.2348	4.5223	1.6963	2.3604	2.4844	-2.44
2	C	57	Glutamine	Glycine	6.72	-45.2°, -49.8°	1.2514	4.5213	1.6962	2.3607	2.4844	-3.35
3	C	75	Asparagine	Tryptophan	49.86	-153.0°, -2.4°	1.2515	4.5218	1.6953	2.3607	2.4844	-2.57
4	C	82	Arginine	Lysine	27.51	-54.9°, -37.4°	1.2464	4.5476	1.6888	2.3585	2.4811	-0.62
5	C	99	Glutamine	Histidine	12.88	-62.1°, -45.7°	1.2516	4.5217	1.6930	2.4607	2.4843	-1.62
6	C	133	Arginine	Cysteine	24.45	-145.4°, 159.8°	1.2514	4.5218	1.6962	2.3601	2.4833	-1.97

The change in the dimensions of the dimer maybe one of the potential reasons that point mutation at ASP78 abolishes the nucleocapsid formation and envelopment. On comparing the parameters obtained in table 5 with those of wild-type HBc protein dimer. It can be seen that the overall dimension of the spike increases by .1722 nm. It indicates that change in overall dimension of .1722 nm can lead to abolishment of nucleocapsid envelopment. But in our point mutation studies, dimensional changes up to .25 nm has been achieved and recorded (table 6). As it can be observed from table 5, the differences in parameters are always below .065 nm.

Whereas it is observed from table 6 that almost all mutations produce equivalent and in many cases greater change than the change produced by point mutation at D78R. In table 6, Δ(parameter A,B,C,D,E) represent the change in dimension of that parameter (in Å) between the mutated HBc capsid protein dimer and the dimer present in wild-type HBV virion. It has been shown that structural way that the affinity of the nucleocapsid to the envelop enables control of envelopment and formation [9]. Moreover, point mutation in ASP78 abolished nucleocapsid envelopment and formation [10]. From the above two facts it is clear that differences as small as 0.065 nm influences the suggested morphology and possibly the functionality of HBV in terms of nucleocapsid envelopment and formation. It is understood from table 6 that the point mutations taken in this paper produce structural changes for the HBc protein dimer by changing the position of the tip. The dimension of the spike determines the point of contact of the dimeric spike with the HBs protein. Hence the change in the dimension of the spike also varies the point of contact of the dimeric spike with HBs protein. It can be seen that the dimension of the spike contributes towards the functional attributes of the HBV.

Table 4. Results obtained for point mutation D78W on chain C.

S.No	Parameter	Amino Acid	Chain	Length
1	A	27 - 35	C	1.2516 nm
2	B	48 - 78	C	4.5300 nm
3	C	82 - 94	C	1.6877 nm
4	D	94 - 110	C	2.3605 nm
5	E	112 - 128	C	2.4843 nm

4 CONCLUSION

In this computational approach for determining the structural basis for stability of the capsid protein, nanoscale structural changes have been observed in accordance with the point mutations made. It has been shown with HBV as an example. Nano-environmental energetics helped us in determining the dimensional changes of the alpha helical

Table 5. Comparison of parameters between wild-type HBV and point mutated (D78W) HBV on chain C with difference in parameters.

S.No	Parameter	Wild-type HBV dimer	D78R	Difference in parameter
1	A	1.2050 nm	1.2516 nm	0.0466 nm
2	B	4.4745 nm	4.5300 nm	0.0555 nm
3	C	1.7443 nm	1.6877 nm	0.0566 nm
4	D	2.2969 nm	2.3605 nm	0.0636 nm
5	E	2.4231 nm	2.4843 nm	0.0631 nm

region after point mutation. This study has further shown that experimental HBc protein point mutations change the morphological and possibly the functional property of HBV. It is important to observe these experimental point mutations in the HBV obtained from patients. Based on these observations, therapeutic molecules can be designed to treat HBV infection.

This study further strengthens the fact that structure of the protein is possibly related to its functionality. Nano-scale dimensionality changes of the alpha helical region have been observed with the help of nano-environmental energetics after point mutation. Computational nanotechnology has been successfully used to determine the

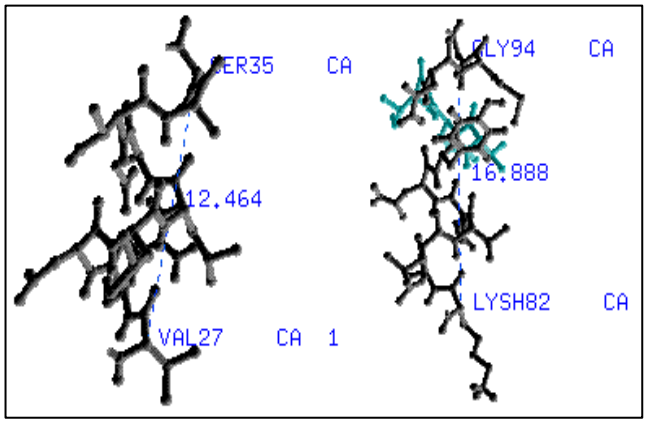

Figure 2: Screenshot providing data for point mutation R82K on chain C. Parameter A (left) and parameter C.

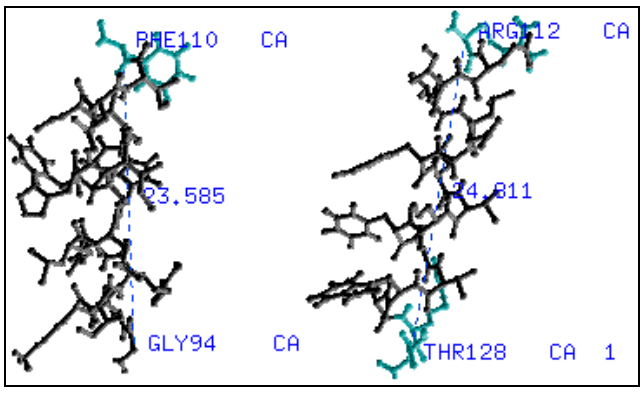

Figure 3: Screenshot providing data for point mutation R82K on chain C. Parameter D (left) and parameter E.

Table 6. Change in parameters for point mutations done on chain C.

Point Mutation	Chain	ΔA (in nm)	ΔB (in nm)	ΔC (in nm)	ΔD (in nm)	ΔE (in nm)
R39V	C	.0298	.0478	.0480	.0635	.0613
Q57G	C	.0464	.0468	.0481	.0638	.0613
N75W	C	.0465	.0473	.0490	.0638	.0613
R82K	C	.0414	.0731	.0555	.0616	.0580
Q99H	C	.0466	.0472	.0513	.0638	.0612
R133C	C	.0464	.0473	.0481	.0632	.0602

key geometric domains contributing towards the structural stability of capsid protein with HBV as an example. Laboratory experiments have to be done to confirm this study, which may provide information on the utility of the changes in the diagnostic and therapeutic applications.

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