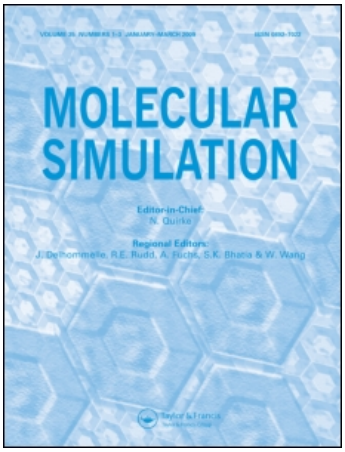

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Quantitative analysis of hepatitis B virion stability upon single-site amino acid mutation based on computational nanotechnology

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Quantitative analysis of hepatitis B virion stability upon single-site amino acid mutation based on computational nanotechnology

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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, molecular energetics of mutated hepatitis B capsid protein dimer was studied for determining the stability of the virus upon single-site amino acid mutation. Results provide structural information regarding the key residues contributing to hepatitis B virion stability and synthesis. This work illustrates the salience of computational nanotechnology and paves the way for future pharmaceutical applications aimed at the destabilisation of the capsid–surface protein interactions.

Keywords: single-site amino acid mutation; hepatitis B capsid protein; structure function relationship; computational nanotechnology; molecular energetic

1. Introduction

A variety of computational tools exists for molecular and structural analyses that can perform amino acid mutations and calculate H-bonds and distances between atoms quite easily [1]. Many bioinformatics tools are available to analyse structures in molecular level, to study the different interactions in nanoscale and to determine the energy in this molecular environment. The different interactions that exist at this level are charge–charge interaction, hydrophilic–hydrophobic interaction, steric effects and bond stability [2]. Structure function relationship of proteins has been studied and structure has been shown to be closely related to the functionality of the protein [3]. In virions, the capsid proteins are nanoscale 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 42 nm [4]. The major building block of the HBV is the core protein (hepatitis B capsid, HBc). The core protein contains 143 amino acids. Previous work has been done to record the dimensions of the HBc experimentally [5,6]. Two bundles, each of length 42 Å, 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 that have formed the dimensions of the spike [7]. The total length of the alpha-helical spike region is 130.425 Å for chain A and 129.545 Å for chain B, which correspond to 94 amino acids. The dimensions of the seven alpha-helical domains are taken as the seven parameters of the HBc protein dimers. The tips of the dimer are in contact with the envelope protein via protrusions that emerged from the inner surface of the envelope [8]. Figure 1 shows HBV particle and surface antigen and ribbon representation of the $T = 4$ HBc, viewed down the icosahedral threefold axis [6].

2. Methods and methodology

2.1 Measurement of parameters

All measurements and molecular energetics computation were carried out in the Swiss-PdbViewer [9], a bioinformatics tool used for computational analysis. Initially the seven parameters as shown in Figure 2 are measured and recorded for the wild-type HBc protein dimer (Tables 1 and 2) for chains *A* and *B*, respectively. Then the single-site amino acid mutations, as given in Tables 3 and 4, are induced in the required chains (chains *A* and *B*), in their appropriate positions. These two tables also give information regarding the solvent accessibility of that particular amino acid selected for mutation and its torsion angle. After this, hydrogen bonds are computed for the dimmers with their molecular surface. Electrostatic potential is then computed and the map is obtained. Coulomb's method is used for the computation of the electrostatic potential. Force field energy computation is then performed and the result is obtained containing the

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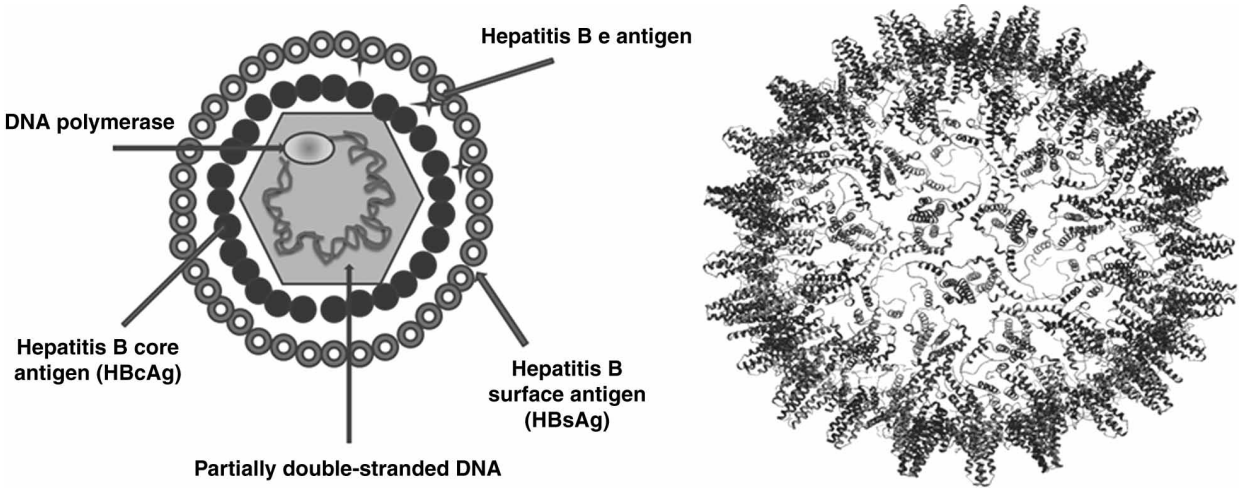

Figure 1. A simplified drawing of the HBV particle and surface antigen (left) and ribbon representation of the $T = 4$ HBc, viewed down the icosahedral threefold axix [6].

total energy with all the energy contributing factors. The mutated dimer in this stage is in its most stable form.

The above-mentioned steps are the prerequisites for running energy minimisation. Energy minimisation is then carried out for the entire structure containing the four dimers. The basic idea of energy minimisation is that a stable state of a molecular system should correspond to a local minimum of their potential energy. This kind of calculation generally starts from an arbitrary state of molecules, and then the mathematical procedure of optimisation allows us to move atoms in a way so as to reduce the net forces (the gradients of potential energy) to nearly zero. The minimised structure has small forces on each atom and therefore serves as an excellent starting point for molecular dynamics simulations.

Energy minimisation for each mutation consists of 500 iterations of the steepest descent followed by 500 iterations of conjugate gradient and repeated till a point, where energy minimisation does not alter the structure of the molecule further. After this step, the final structure is obtained. After obtaining the final structure, the seven parameters are measured and recorded as a table with their atom pair potential in kcal/mol (Tables 5 and 6). The ΔG_a value forms a very important factor in deciding the amino acid to replace the native amino acid in the capsid protein.

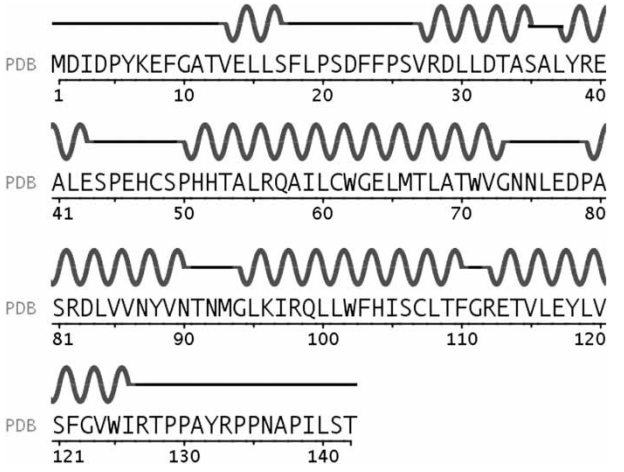

Figure 2. HBc protein amino acid sequence where the curved lines above the sequence represent the alpha-helical region of the protein. There are a total of seven alpha-helical regions that make up 94 residues of the total of 143 residues (i.e.) 66% of the total. The numbers below the amino acid sequence represents residue id. The alpha-helical regions are taken as the parameters of comparison.

2.2 Energy contributing factors

The objective of molecular mechanics is to predict the energy associated with a given conformation of a molecule. However, molecular mechanics energies have no meaning as absolute quantities. Only differences in energy between two or more conformations have meaning. A simple molecular

Table 1. Dimension of the alpha-helical region for chain *A* of the HBc protein, which is also taken as the parameter to compare the change in its dimension upon single-site amino acid mutation.

S.no.	Parameter	Amino acid sequence ID	Length in Å
1	A	13–17	6.347
2	B	27–37	15.365
3	C	38–43	7.957
4	D	49–73	36.128
5	E	79–90	15.515
6	F	92–110	26.808
7	G	112–127	22.305

Table 2. Dimension of the alpha-helical region for chain *B* of the HBc protein, which is also taken as the parameter to compare the change in its dimension upon single-site amino acid mutation.

S.no.	Parameter	Amino acid sequence ID	Length in Å
1	A	13–17	5.713
2	B	27–37	15.224
3	C	38–43	8.311
4	D	49–73	35.973
5	E	79–90	15.622
6	F	92–110	26.912
7	G	112–127	21.790

mechanics energy equation is given by [10]:

$$\text{Total Energy} = \text{Stretching Energy} + \text{Bending Energy} + \text{Torsion Energy} + \text{Non-Bonded Interaction Energy}.$$

2.2.1 Stretching energy

$$E = \sum_{\text{bonds}} k_b(r - r_o)^2.$$

The stretching energy equation is based on Hooke's law. The 'k_b' parameter controls the stiffness of the bond spring, while 'r_o' defines its equilibrium length. Unique 'k_b' and 'r_o' parameters are assigned to each pair of bonded atoms based on their types (C–C, C–H, O–C, etc.). This equation estimates the energy associated with vibration about the equilibrium bond length. This is the equation of a parabola.

2.2.2 Bending energy

$$E = \sum_{\text{angles}} k_\theta(\theta - \theta_o)^2.$$

The bending energy equation is also based on Hooke's law. The 'k_θ' parameter controls the stiffness of the angle spring, while 'θ_o' defines its equilibrium angle. This equation estimates the energy associated with vibration about the equilibrium bond angle. Unique parameters for angle bending are assigned to each bonded triplet of atoms based on their types (C–C–C, C–O–C, C–C–H, etc.). The effect of the 'k_b' and 'k_θ' parameters is to broaden or steepen the slope of the parabola. The larger the value of 'k', the more energy is required to deform an angle (or bond) from its equilibrium value. Shallow potentials are achieved for 'k' values between 0.0 and 1.0.

2.2.3 Torsion energy

$$E = \sum_{\text{torsions}} [1 + \cos(n\tau - \phi)].$$

The torsion energy is modelled by a simple periodic function. The torsion energy in molecular mechanics is primarily used to correct the remaining energy terms rather than to represent a physical process. The torsion energy represents the amount of energy that must be added to or subtracted from the Stretching Energy + Bending Energy + Non-Bonded Interaction Energy terms to make the total energy agree with the experiment or rigorous quantum mechanical calculation for a model dihedral angle. The 'A' parameter controls the amplitude of the curve, the 'n' parameter controls its periodicity and 'ϕ' shifts the entire curve along the rotation angle axis (τ). The parameters are determined from curve fitting. Unique parameters for torsional rotation are assigned to each bonded quartet of atoms based on their types (C–C–C–C, C–O–C–N, H–C–C–H, etc.).

2.2.4 Non-bonded interaction energy

$$E = \sum_i \sum_j \frac{A_{ij}}{r_{ij}^6} + \frac{B_{ij}}{r_{ij}^{12}} + \sum_i \sum_j \frac{q_i q_j}{r_{ij}}.$$

The non-bonded energy represents the pair-wise sum of the energies of all possible interacting non-bonded atoms i and j. The first term is the van der Waals term and the second term is electrostatic term. The 'r_{ij}' parameter represents the distance between the atoms and 'q' parameter represents the charge on the atom.

Table 3. Set of considered single-site amino acid mutation in chain *A*.

S.no.	Sequence position	Mutated residue	Introduced mutation	Chain	Solvent accessibility in %	Torsion angle (ϕ,ψ)
1	14	Glutamine	Histidine	A	79.6	−58.4°, −48.1°
2	28	Arginine	Proline	A	62.3	−42.4°, −78.6°
3	39	Arginine	Lysine	A	39.7	−38.5°, −64.6°
4	70	Threonine	Glycine	A	65.6	−75.9°, −70.4°
5	87	Asparagine	Glycine	A	65.6	−101.8°, −54.4°
6	92	Asparagine	Glycine	A	52.6	−73.7°, −57.7°
7	127	Arginine	Glycine	A	66.8	−76.8°, 4.1°

Table 4. Set of considered single-site amino acid mutation in chain *B*.

S.no.	Sequence position	Mutated residue	Introduced mutation	Chain	Solvent accessibility in %	Torsion angle (ϕ,ψ)
1	14	Glutamine	Lysine	B	72.7	−55,7°, −35.9°
2	28	Arginine	Proline	B	59.8	−53.2°, −76.8°
3	39	Arginine	Aspartic acid	B	27.1	−43.1°, −75.0°
4	60	Leucine	Threonine	B	26.2	−79.6°, −52.0°
5	90	Asparagine	Glycine	B	53.3	−51.6°, −18.1°
6	99	Glutamine	Histidine	B	20.2	−41.5°, −68.9°
7	121	Serine	Glycine	B	56.3	−70.3°, −33.0°

The non-bonded energy accounts for repulsion, van der Waals attraction and electrostatic interactions. The van der Waals attraction occurs at short range, and rapidly dies off as the interacting atoms move apart by a few Angstroms. Repulsion occurs when the distance between interacting atoms becomes even slightly less than the sum of their contact radii. Repulsion is modelled by an equation that is designed to rapidly blow up at close distances. The energy term that describes attraction/repulsion provides for a smooth transition between these two regimes. The '*A*' and '*B*' parameters control the depth and position (interatomic distance) of the potential energy for a given pair of non-bonded interacting atoms (C:C, O:C, O:H, etc.). In effect, '*A*' determines the degree of 'stickiness' of the Van der Waals attraction and '*B*' determines the degree of 'hardness' of the atoms.

3. Results

3.1 Parameter selection

The dimensions of the seven alpha-helical regions are taken as the seven parameters of the HBc protein dimers. These alpha-helical regions are chosen as the parameters because small changes in the dimension of these parameters, in the order of 0.2 Å, were found to alter the structure of the capsid dimer significantly. The HBc protein consists of four chains, namely *A*, *B*, *C* and *D*. Two chains, *A* and *B*, are taken for the comparative study.

3.2 Native amino acid selected to perform single-site mutation

To perform single-site amino acid mutation analysis, 14 mutations were selected. The amino acid to replace the native ones is decided based upon the following rule. The overall stability of the protein upon single-site amino acid mutation is calculated from atom pair potentials and torsion angle potentials. The atom pair potential that is used to predict the overall stability of the virion upon single-site mutation was calculated mathematically using the following formula [11]:

$$\Delta G_a = -kT \ln\left[\frac{g_{ij}(r_d)}{g(r_d)}\right],$$

where k represents the Boltzmann constant ($1.38 \times 10^{-23}\,\mathrm{m^2\,kgs^{-2}\,K^{-1}}$), T the temperature, $g_{ij}(r_d)$ the radial pair distribution function of a pair (i,j) separated by a distance and $r_d{\cdot}g(r_d)$ is the description of the reference state. A positive value of ΔG_a represents a stabilising mutation, whereas a negative value of ΔG_a represents a destabilising mutation.

The torsion angle potential that is used to predict favourable or unfavourable torsion of a single-site mutation was calculated mathematically using the following formula [11]:

$$\Delta G_{\mathrm{tor}} = -kT \ln\left[\frac{g(\phi,\psi)}{g_{\mathrm{ref}}(\phi,\psi)}\right],$$

where k represents the Boltzmann constant ($1.38 \times 10^{-23}\,\mathrm{m^2\,kgs^{-2}\,K^{-1}}$), T the temperature, $g(\phi,\psi)$ and $g_{\mathrm{ref}}(\phi,\psi)$ are torsion angle distribution of a specific amino acid and the average distribution over all the amino acids, respectively. Similar to atom pair potential, a positive value of ΔG_{tor} represents a favourable torsion angle, whereas a negative value of ΔG_{tor} represents an unfavourable torsion angle. In the case of unfavourable torsion angles, the atom potentials may have higher impact on stability, which results in a stabilising mutation.

3.3 Amino acid selected to replace the native amino acid

The amino acids selected to replace the native amino acid on the capsid dimer on chains *A* and *B* are listed in Tables 3 and 4, respectively. The table presents data on the amino acids present in wild-type HBc protein dimer before mutation, with their chain, the position in their respective chains, and the amino acid with which single-site mutation is performed. The table also provides information on the solvent accessibility and the torsion angle of the particular amino acid.

The amino acid in the native HBc protein selected to perform the single-site amino acid mutation is based upon the following factors. The accessibility of solvent to the site of the mutation plays an important role. The better the accessibility of solvent, the easier the mutation can be performed [12]. Figure 3 represents the accessibility of solvent versus residue ID for chains *A* and *B*.

Table 5. Change in the dimension of the selected parameter (chain *A*) upon the considered single-site amino acid mutation.

S.no.	Induced aingle-site amino acid mutation	Parameter *A* in Å	Parameter *B* in Å	Parameter *C* in Å	Parameter *D* in Å	Parameter *E* in Å	Parameter *F* in Å	Parameter *G* in Å	Predicted ΔG_a value (Kcal/mol)
1	E14H	6.555	15.635	8.605	35.145	16.294	27.500	22.697	− 1.51
2	R28P	6.551	15.658	8.605	36.559	16.229	27.497	22.668	− 2.23
3	R39K	6.548	15.628	8.542	36.560	16.295	27.501	22.671	− 1.71
4	T70G	6.550	15.633	8.606	36.561	16.293	27.500	22.670	− 1.03
5	N87G	6.549	15.631	8.609	34.800	16.294	27.502	22.672	− 2.43
6	N92G	6.411	15.392	8.458	36.356	15.826	27.480	22.485	− 2.41
7	R127G	6.544	15.631	8.608	36.559	16.296	27.503	22.534	− 0.79

The performed mutations are encircled and the native amino acid and the replacing amino acid are also represented in the figure. The force field energy calculation on HBc protein is another factor. The more negative the value of the total energy contributed by an amino acid, the more it contributes towards the stability of the protein structure. Amino acids that contribute the maximum towards the stability of the protein are always selected and it is ensured that the selected amino acid lies on a secondary structure of the dimers. It is generally observed that amino acids that lie on secondary structures, when replaced, while all other factors are favourable, cause maximum change to the structure of the protein [13]. Hence, we choose an amino acid that is most accessible while it has the least total energy contribution to the structure as a whole and lies on a secondary structure. It is always ensured that the replacing amino acid causes maximum structural change for the HBc protein dimer. Chains *A* and *B* were chosen for the study since the AB-dimers formed the most intimate contacts with the hepatitis B envelope protein [8].

3.4 Interpretation of result

After mutations were performed on amino acids given in Table 3 on chain *A* and amino acid in Table 4 on chain *B*, the following results were obtained. The results for mutations performed in chains *A* and *B*, respectively, have been provided in Tables 5 and 6. The recorded results are under the following characteristics:

- chain
- residue ID
- amino acid present in wild-type HBV dimer
- amino acid with which mutation is performed
- the seven parameters – *A*, *B*, *C*, *D*, *E*, *F* and *G* (in Å)

Figure 4(a),(b) depicts the change in dimension of parameters (except *G*) upon single-site amino acid mutation R28P in chain *B*. Comparing the value obtained in Table 1 for chain *A* with the parameters obtained after single-site mutation in Table 5, the following observations were made. The maximum change in the seven parameters combined together $(A + B + C + D + E + F + G)$ is obtained for the mutation N87G (4.288 Å) followed by E14H (3.972 Å) and then T70G (3.388 Å). Similarly, comparing the value obtained in Table 2 for chain *B* with the parameters obtained after mutation in Table 6, the following observations were made. The maximum change in the seven parameters combined together $(A + B + C + A + B + C + D + E + F + G)$ is obtained for the mutation R28P (3.79 Å) followed by S121G (3.678 Å) and then R39D (3.66 Å). The combined value of the seven parameters in wild-type HBc dimer protein, for chain *A* is 130.425 Å and for chain *B* is 129.545 Å. The difference in value of the seven parameters combined together for chain

Table 6. Change in the dimension of the selected parameter (chain *B*) upon the considered single-site amino acid mutation.

S.no.	Induced aingle-site amino acid mutation	Parameter *A* in Å	Parameter *B* in Å	Parameter *C* in Å	Parameter *D* in Å	Parameter *E* in Å	Parameter *F* in Å	Parameter *G* in Å	Predicted ΔG_a value (Kcal/mol)
1	E14K	6.045	15.785	8.870	36.824	16.298	27.100	22.247	− 1.58
2	R28P	6.069	15.899	8.886	36.826	16.313	27.101	22.241	− 2.20
3	R39D	6.054	15.796	8.871	36.822	16.301	27.110	22.251	− 1.78
4	L60T	6.059	15.801	8.875	36.828	16.296	27.098	22.245	− 3.94
5	N90G	6.060	15.842	8.869	36.819	16.293	27.089	22.240	− 3.21
6	Q99H	6.055	15.831	8.866	36.821	16.295	27.091	22.243	− 1.90
7	S121G	6.040	15.855	8.864	36.825	16.292	27.101	22.246	− 1.25

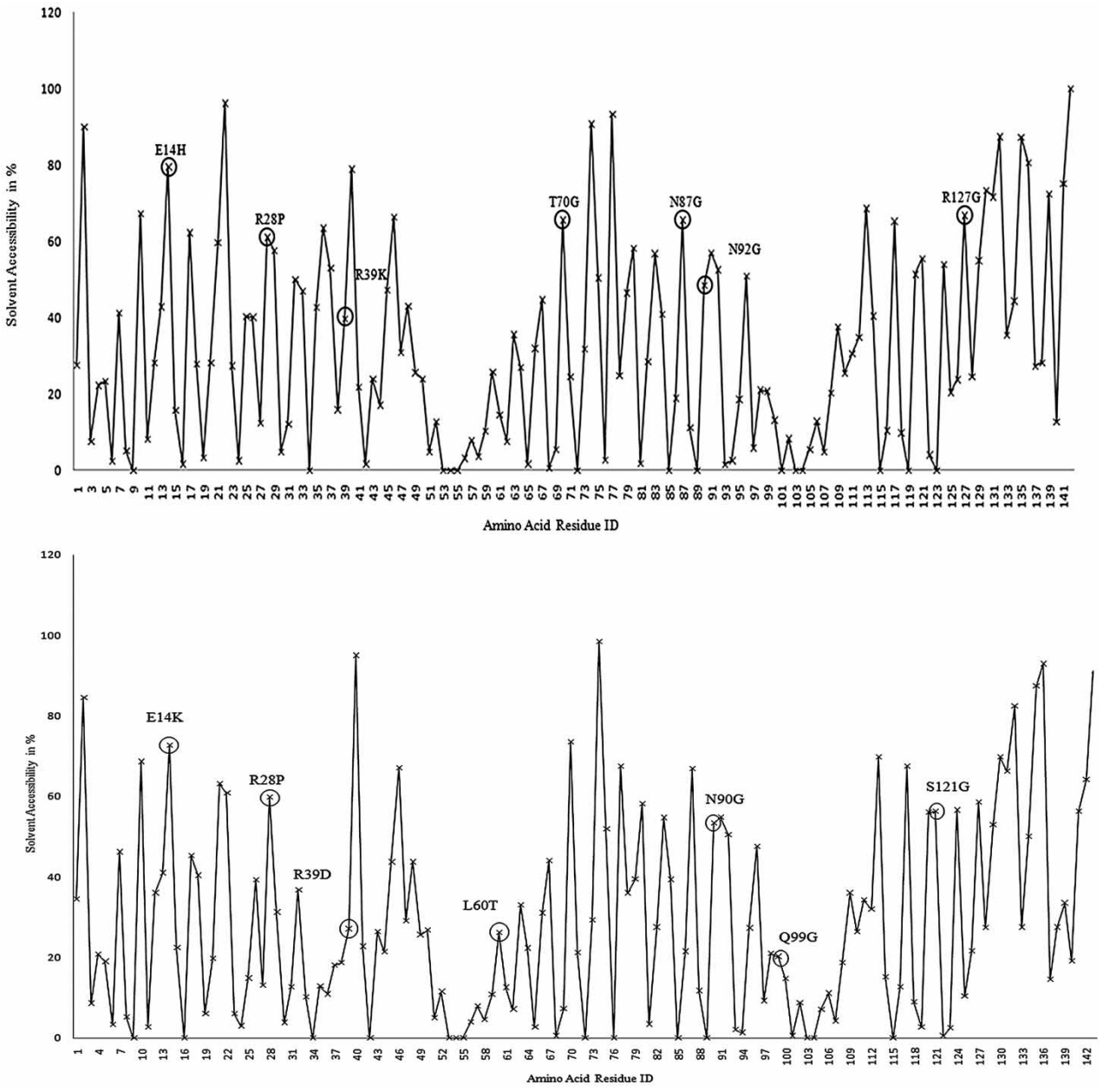

Figure 3. Plot of accessibility of solvent in % versus amino acid residue ID for chains *A* (top) and *B* (bottom) of the HBc protein. The selected residues for single-site amino acid mutation have been circled and the performed mutations are also mentioned.

A between mutated and wild-type HBc dimers varies between 1.632 and 3.342 Å. Similarly, the difference in value of the seven parameters combined together for chain *B* between mutated and wild-type HBc dimers varies between 3.624 and 3.79 Å. These seven 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 1–4 Å, it has a significant effect on the structural and functional effects of the HBV virion as discussed below.

4. Discussion

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 [8]. The exact position of the contact area on the spike and the size of area of contact vary between the two morphological phenotypes (compact and gapped). 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.

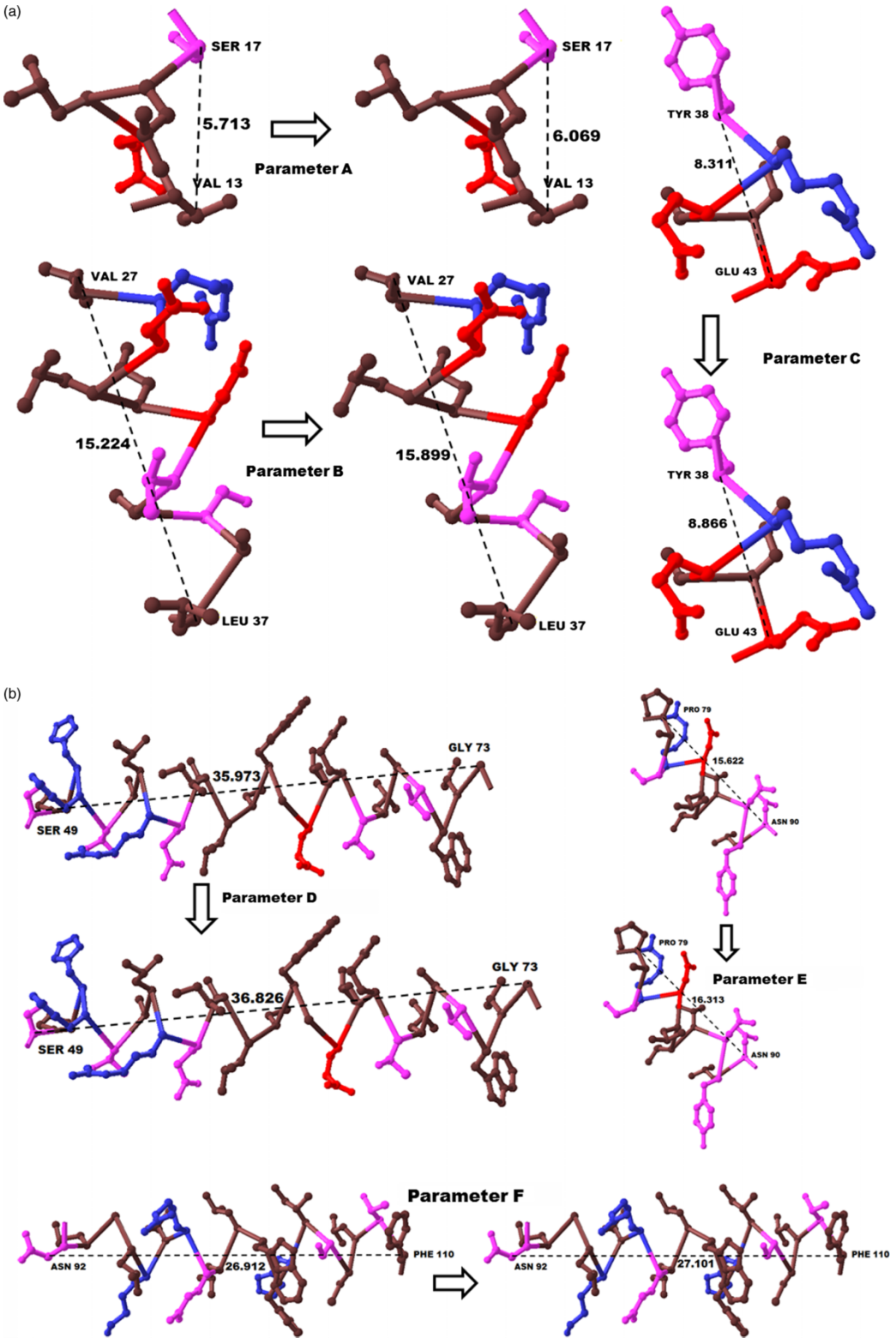

Figure 4. (a) Depicts the change in dimension of parameters *A*, *B* and *C* upon single-site amino acid mutation R28P in chain *B*. It shows the dimension of the parameter in wild-type HBc and then the dimension of the same parameter after mutation with acidic groups in red, basic groups in blue, polar groups in pink and hydrophobic groups in brown. (b) Depicts the change in dimension of parameters *D*, *E* and *F* upon single-site amino acid mutation R28P in chain *B*. It shows the dimension of the parameter in wild-type HBc and then the dimension of the same parameter after mutation with acidic groups in red, basic groups in blue, polar groups in pink and hydrophobic groups in brown.

It has been found out that the 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 [8]. In the single-site mutation analysis in this paper, the dimensions of the spike vary in accordance with the various 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 HBV.

It has been found by experimental methods that single-site mutation in ASP78 abolished nucleocapsid formation completely [13]. It has been shown that ASP78 is a potential site of contact between the HBc protein dimeric spike and HBs protein in both the chains (*A* and *B*) [8]. In our bioinformatics study, mutation is performed in ASP78 with arginine to see the change in the seven parameters (Tables 7, 8 and 9). Arginine was selected to perform the mutation since it had negative value of ΔG_a (-1.60 kcal/mol) and hence destabilising. Any other amino acid having negative value of ΔG_a could have been selected, but arginine was found to produce the maximum structural change. The change in the dimensions of the dimer may be one of the potential reasons that mutation at ASP78 abolishes the nucleocapsid formation and envelopment.

Tables 7 and 9 represent the dimension of the seven parameters in wild-type HBc protein and modified parameters after single-site amino acid mutation D78R in chains *A* and *B*, respectively. It can be seen that the overall dimension of the spike increases by 3.258 Å for chain *A* and 3.676 Å for chain *B*. It indicates that change in overall dimension of 3.258 and 3.676 Å in chains *A* and *B*, respectively, can lead to the abolishment of nucleocapsid envelopment. But in our mutation studies, dimensional changes from 1.983 to 4.288 Å have been achieved and recorded (Tables 11 and 12). Individual parameter differences have been shown in Tables 8 and 10 for D78R and Tables 11 and 12 for all single-site mutations that produced the maximum individual parameter change of all possible mutations. In Tables 11 and 12, Δ(parameter *A*, *B*, *C*, *D*, *E*) represents 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 changes at the tip may alter the charge distribution in such a way that the affinity of the nucleocapsid to the envelop enables the control of envelopment and formation [8] and mutation in ASP78 abolished nucleocapsid envelopment and formation [13]. From the above two facts, it is clear that differences as small as 2 Å influence the suggested morphology and possibly the functionality of HBV in terms of nucleocapsid envelopment and formation. Hence, it is understood from Tables 8 and 10 that the mutations taken in this paper produce structural changes for the HBc protein dimer by changing the position of the tip. The dimension of the seven parameters 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 directly or indirectly contribute towards the functional attributes of the HBV. These facts further strengthen the purpose of our study.

5. Conclusion

In this bioinformatics approach towards identifying amino acids for single-site mutation in HBc protein to prevent interaction of the virus' protein core with its envelope, structural changes have been observed in accordance with the mutation made. The mutations in the spiking region were studied for changes in the key geometric parameters. These parameters (the lengths of the alpha-helical regions of the spike), show significant translation after mutation. It has been shown with an experimental single-site amino acid mutation (D78) [13] that a dimensional difference as small as 2 Å can completely change the functionality of the HBV leading to a lack of nucleocapsid envelopment and nucleocapsid formation. This study has shown that HBc protein single-site amino acid mutations change the morphological and possibly the functional property of HBV. Molecular energetics measurements helped us in determining the dimensional changes of the alpha-helical region after mutation.

This study further strengthens the fact that structure of the protein is possibly related to its functionality. Computational nanotechnology has been successfully used

Table 7. Results obtained for the dimension of the parameters in chain *A* upon single-site amino acid mutation in position 78 (D78R).

S.no.	Parameter	Amino acid sequence ID	Chain	Length in Å
1	A	13–17	A	6.551
2	B	27–37	A	15.633
3	C	38–43	A	8.604
4	D	49–73	A	36.564
5	E	79–90	A	16.167
6	F	92–110	A	27.495
7	G	112–127	A	22.669

Table 8. Comparison of parameters between wild-type HBc protein and single-site amino acid mutated HBc capsid for chain *A* (D78R).

S.no.	Parameter	Dimension of wild-type HBc protein in Å	Dimension of single-site amino acid mutated HBc protein in Å	Difference between parameter in Å
1	A	6.347	6.551	0.204
2	B	15.365	15.633	0.268
3	C	7.957	8.604	0.647
4	D	36.128	36.564	0.436
5	E	15.515	16.167	0.652
6	F	26.808	27.495	0.687
7	G	22.305	22.669	0.364

Table 9. Results obtained for the dimension of the parameters in chain *B* upon single-site amino acid mutation in position 78 (D78R).

S.no.	Parameter	Amino acid sequence ID	Chain	Length in Å
1	A	13–17	B	6.065
2	B	27–37	B	15.786
3	C	38–43	B	8.873
4	D	49–73	B	36.824
5	E	79–90	B	16.331
6	F	92–110	B	27.101
7	G	112–127	B	22.241

Table 10. Comparison of parameters between wild-type HBc protein and single-site amino acid mutated HBc capsid for chain *B* (D78R).

S.no.	Parameter	Dimension of wild-type HBc protein in Å	Dimension of single-site amino acid mutated HBc protein in Å	Difference between parameter in Å
1	A	5.713	6.065	0.352
2	B	15.224	15.786	0.562
3	C	8.311	8.873	0.562
4	D	35.973	36.824	0.851
5	E	15.622	16.331	0.709
6	F	26.912	27.101	0.189
7	G	21.790	22.241	0.451

Table 11. Change in the parameters for the single-site mutations done in chain *A*.

Single-site mutation	Chain	ΔA (in Å)	ΔB (in Å)	ΔC (in Å)	ΔD (in Å)	ΔE (in Å)	ΔF (in Å)	ΔG (in Å)	Total change in parameter (in Å)
E14H	A	0.208	0.27	0.648	0.983	0.779	0.692	0.392	3.972
R28P	A	0.204	0.293	0.648	0.431	0.714	0.689	0.363	3.342
R39K	A	0.201	0.263	0.585	0.432	0.78	0.693	0.366	3.32
T70G	A	0.203	0.268	0.649	0.433	0.778	0.692	0.365	3.388
N87G	A	0.202	0.266	0.652	1.328	0.779	0.694	0.367	4.288
N92G	A	0.064	0.027	0.501	0.228	0.311	0.672	0.18	1.983
R127G	A	0.197	0.266	0.651	0.431	0.781	0.695	0.229	3.25

to determine the key geometric domains contributing towards the structural stability of capsid protein. Laboratory experiments have to be done to confirm this study, which may provide information on the utility of the changes in the diagnostic applications. It is important to observe these mutations experimentally in the HBV obtained from patients. Based on these observations, therapeutic molecules can be designed to treat HBV infection.

Table 12. Change in the parameters for the single-site mutations done in chain *B*.

Single-site mutation	Chain	ΔA (in Å)	ΔB (in Å)	ΔC (in Å)	ΔD (in Å)	ΔE (in Å)	ΔF (in Å)	ΔG (in Å)	Total change in parameter (in Å)
E14K	B	0.332	0.561	0.559	0.851	0.676	0.188	0.457	3.624
R28P	B	0.356	0.675	0.575	0.853	0.691	0.189	0.451	3.79
R39D	B	0.341	0.572	0.56	0.849	0.679	0.198	0.461	3.66
L60T	B	0.346	0.577	0.564	0.855	0.674	0.186	0.455	3.657
N90G	B	0.347	0.618	0.558	0.846	0.671	0.177	0.45	3.667
Q99H	B	0.342	0.607	0.555	0.848	0.673	0.179	0.453	3.657
S121G	B	0.327	0.631	0.553	0.852	0.67	0.189	0.456	3.678

Notes

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