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Full Length Research Paper

Cyclo[(-D-Gly$_3$-L-Asp$_3$)] in combination with C and D polypeptide chains of NS3; suggestion a novel nanoparticle to stimulate immune system against hepatitis C

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Development of a hepatitis C vaccine is a challenge. Although several vaccines are currently under development, no effective vaccine is currently available. Non structural protein- NS3- can stimulate TH1 and make enhancement in NS3-specific interferon-γ (IFN-γ) serum level. It is thought to use nanoparticles which are biodegradable and safe to body can be a novel approach to deliver epitops as vaccine. Adding the polypeptides components of NS3 on cyclic hexa peptide nanorings with the ability to stimulate immune system without any risk of infection is the main goal of this research. In present study, the nano structures were designed using Hyperchem™ 8.0.6 software and ArgusLab 4.0.1 package. *Cyclo* [(-D-G$_3$-L-D$_3$)] nanoring has been studied by quantum mechanical calculations within the Onsager self- consistent reaction field model at room and critical body temperatures, using Gaussian 03 package. Radius of gyration and φ and ψ rotation were analyzed with VMD 1.8.2. Montecarlo molecular mechanic method in both MM+ and Bio+ (Charmm) force fields were operated in 200pSec. 1, 2 and 3 polypeptide chains of C and D, separated from NS3 of protease (PDB ID: 1NS3), were substituted to the core. It was revealed that by increasing the polypeptide substituent, the potential energy was increased in systems. The mean of relative potential in *Cyclo* [(-D-G$_3$-L-D$_3$)] substituted with D-polypeptide chain was about two times more stable than the one of C- polypeptide chain, in the same condition. Φ and ψ, changes due to temperature arising were approved in these cases. Although both C and D polypeptide chains were stable in water medium (such as body condition), the results revealed that D chain is more stable to use for nanovaccine fabrication. The *Cyclo* [(-D-G$_3$-L-D$_3$)] with three substituent of D-chain is suggested, based on results.

Key words: Cyclo[(-D-Gly3-L-Asp3)], non structural protein- NS3, vaccine, hepatitis C, Ramachandran plot.

INTRODUCTION

"About 130 to 170 million people are chronically infected with hepatitis C virus (HCV), and more than 350000 people die from hepatitis C-related liver diseases each year. Despite ongoing research, there is currently no vaccine to prevent hepatitis C virus infection" (World Health Organization. 2011). HCV is a Flaviviridae member. It is a small virus enveloped with a single-stranded positive RNA genome inside (Kato et al., 1990). Within 7 to 8-week postinfection, it is believed that neutralizing antibodies against HCV could be observed and the clearance of antibodies occurred after (Lanford, et al., 2004).There is no protection against reinfection (Bassett et al, 2001; Lanford et al., 2004; Houghton and Abrignani, 2005). It is revealed that clearance of infection has been associated with a strong T helper 1 T cell response directed against the conserved nonstructural proteins of the virus (Diepolder et al. 1995; Missale et al.,

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1996). Nonstructural proteins of HCV are able to increase, multispecific CD4+ helper and CD8+ cytotoxic T cell response which can clear infection (Cooper et al., 1999). This virus is highly heterogeneous. Up to now, more than eleven HCV genotypes with several distinct subtypes have been identified. Moreover, such heterogeneity makes some difficulties in development of vaccines and global protection (Ohno, 1996; Abrignani et al., 1999; Prince and Shata, 2001; Alberti et al., 2003). Synthetically or genetically engineered antigens are being developed to be utilize as vaccines (Airoldi and Berghella 2006). Th1 immune response against HCV NS3 is very critical and I important in immunization against HCV infection, because of that the development of a vaccine directed against HCV NS3 is considerable. It is found that Anti-HCV/NS3 IgG1 and IgG2a responses were changed in immunized mice (Jiao et al., 2004).

Three structural and six nonstructural (NS) proteins are consists in a polyprotein structure arranged in the sequence, NH2-C-E1-E2-NS2-NS3-NS4A-NS4B-NS5ANS5B- COOH. This protein belongs to the chymotrypsin family and it mediates proteolytic cleavage at the NS4B/5A, NS3A, NS5A/5B, and NS4A/4B junctions (Bartenschlager et al., 1993; Grakoui et al., 1993; Les and Fordham, 1996; Lohmann et al., 1999). The NS3 has been extensively characterized at both biochemical and structural levels (Love et al., 1996; Steinkuhler et al., 1996). Several cell activity such as replicon is reported for NS3. Recombinant chimeric viruses show that propagation requires the activity of NS3 (Cho and Li, 1997; Filocamo et al., 1997). It has RNA helicase activity and exhibits NTPase function, also; the unwinding of RNA–RNA in viral replication is done by that (Palmenberg, 1990).

Peptide -based nanostructures

Peptide building blocks had been introduced for the assembly of nano-ordered material a decade ago when Ghadiri (Jeffrey et al., 1996) was the first to describe a new class of biochemical nanotubes based on rationally designed cyclic polypeptides. These cyclic peptides were produced by an alternating even number of D- and L-amino acids, which interact through noncovalent interactions to an array of selfassembled nanotubes. The internal diameter of the nanotubes ranges between 7-8 Å and can be controlled by changing the number of the amino acids. Various biophysical properties and applications were offered for these circular structures (Khalili et al., 2010a; Khalili and Monajjemi, 2010b)

Liposome co-encapsulated recombinant HCV NS3 were prepared as nanoparticles to stimulate immune system. It is suggested that This suggests that the immune responses induced by free rNS3 protein immunization favor the Th2 pathway.(Jiao et al., 2004)

Direct immuno-stimulation of antigen presenting cells (APC) or/and delivering antigen to specific cellular compartments and promoting antigen uptake by appropriate stimulatory cell types can be performed by particulates. Kalkanidisa and colleagues suggest the particulates mediated immune-stimulation and make antigen presenting cells (APC) to present the antigen in surface. They suggest a method for the preparation of a novel nanoparticle-based antigen delivery system based on the use of 40 nanometer (nm) inert solid carrier beads which induces strong cellular and humoral immune responses in mice and sheep. In this simple system is to which antigen is covalently coupled before injection (Kalkanidisa et al., 2006).To prepare and characterize of chitosan nanoparticles and chitosan-coated emulsions to vaccine delivery also reported (Nagamoto et al., 2004). Cui report importance of utilization of LPD in enhancing the antitumor immunity(Cui et al., 2005).

The aim of this study is to design and computational study, as well as simulation of a combined structure of C and D polypeptide chains of nonstructural protein (NS3), with multifunctional biodegradable cyclic hexa peptide nanoring, and to suggest a novel immune-stimulator against HCV.

COMPUTATIONAL METHOD

The quantum mechanical method that was chosen to geometrically optimize the *Cyclo* [(-D-G_3-L-D_3)] nano rings was that of the Hartree–Fock (HF) equations using atomic orbital basis functions of type STO-3G. The HF method is defined as the most frequently used type of *ab initio* quantum calculation. Its wave function minimizes the molecular energy. The HF Hamiltonian is a function of its own orbital Eigen-functions, and therefore the HF equations are solved self consistently. ANOVA single factor test was used to analyze the variances in SPSS 17.

In present study, the structure of *Cyclo* [(-D-G_3-L-D_3)] nanoring has been studied by quantum mechanical calculations within the Onsager self - consistent reaction field (SCRF) model using a Hartree-Fock method (RHF) at the RHF/STO-3G (5D-7F) level. The structures were designed by Hyperchem^{TM} 8.0.6 software and the geometry of Cyclo hexa peptide nano rings are fully optimized in water at 290 (298.15), 310 and 315K. The entire calculations were performed at Hartree-Fock (HF) levels on a Pentium IV/2.8 GHz personal computer using Gaussian 03W program package, invoking geometry optimization. Geometry generated from standard parameters was minimized without any constraint in the potential energy at Hartree-Fock level, adopting the standard STO-3G (5D-7F) basis set. The A0 value for SCRF calculations based on the Onsager model was calculated for all parameter, separately. using the VMD 1.8.2, the radius of gyration and also Φ and Ψ rotation in back bone of *Cyclo* [(-D-G_3-L-D_3)] nanoring, C-chain and D-chain as well as designated nanovaccine were calculated (Khalili et al., 2010a, Khalili and Monajjemi, 2010b). The effect of a solvent can be incorporated in quantum-chemical calculations most easily by considering it as a continuous dielectric medium, characterized by a dielectric constant. The electric field caused by the molecule induces a polarization of the medium, which in turn acts on the electrons in the molecule (Self- Consistent Reaction Field, SCRF). The model thus contains the quantum-mechanical description of the molecule and a classical medium. In the Gaussian programs, a simple approximation is used in which the volume of the solute is used to compute the radius of a cavity which forms the hypothetical surface of the molecule.

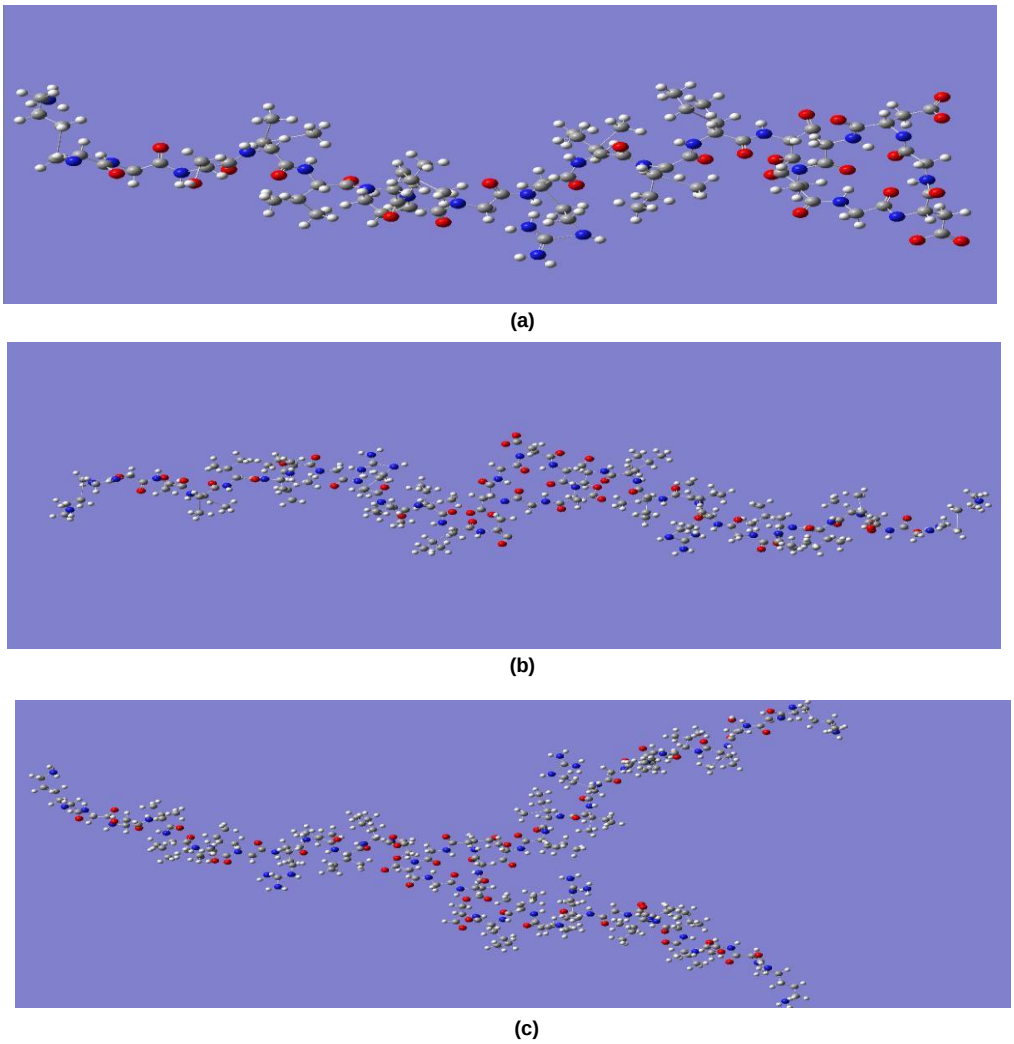


Figure 1. The schematic molecular figures of cyclo[-(D-G3-L-D3)] in combination to C and/or D polypeptide chains. The C and D chains are separated from NS3/NS4 Protease (PDB ID, 1NS3). The figures show one (a), two (b) and three (c) polypeptides.

Montecarlo molecular mechanic method, in both MM+ and Bio+ (Charmm) force fields in 200pSec, was performed to simulate the nanovaccine in three mentioned critical temperatures of room temperature, normal body (310K) and fever temperature (315K).

RESULTS

The protein structure prediction is the prediction of the 3D conformation of a protein, when the sequence residues are known. The 3D conformation of a protein could be modeled involving energy functions to be minimized and constraints on the amino acids positions. During passed years, the global optimizations are used for this purpose such as different classes of methods (*ab-initio modeling*): Genetic algorithms (Holland and Siam, 1973), branch and bound (Nemhauser and Wolsey, 1998), simulated annealing (Kirkpatrick et al., 1983), smoothing methods (Stillinger and Weber, 1988) and constraints (Backofen, 2001). Some additional databases are available and it is possible to employ a different class of methods. The protein is matched against very similar sequences and the conformation prediction exploits this valuable information. A fundamental role in the design of a predictive method is played by the spatial representation of the protein and the static energy function, which is to be at a minimum for native conformations.

In the present study, 1, 2 and 3 polypeptide chains of C and D, extracted from NS3/NS4 of 1NS3 protease, were attached to the core of cyclo hexa peptide nano ring (Figure 1a to c). The relative molecular mechanic potential energy diagrams resulted by Montecarlo revealed that by increasing the polypeptide substituent on core nano ring the potential energy is increased in systems. The trend is different for C and D polypeptide

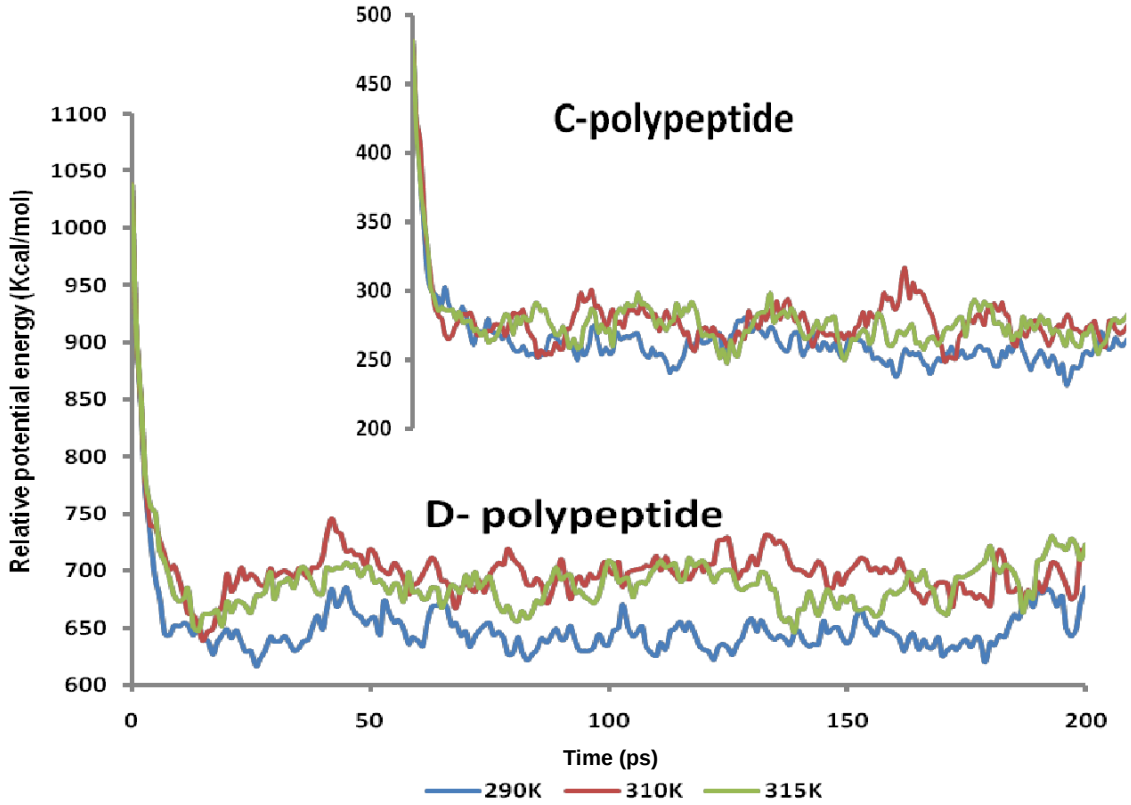


Figure 2. Potential energy comparison of C and D polypeptide after 200 ps Montecarlo simulation.

Table 1. Statistical analysis of mean potential energy (P<0.01).

Parameter	C-polypeptide (K)			D-polypeptide (K)		
	290	310	315	290	310	315
Mean	263.42	279.42	277.48	651.70	701.20	691.69
Std. dev.	24.93	24.13	22.67	40.15	35.05	36.91

substituent (Figure 2). The mean of relative potential in *Cyclo* [(-D-G_3-L-D_3)] substituted by n = 1,D-polypeptide chain is about two times more stable than that one of C-polypeptide chain, in the same condition. The behavior of both polypeptides was similar (Figure 2). The ANOVA single factor test showed that the differences in variances were significant between the means of both polypeptide chains (p<0.01). Although the energy changes were not equal in C and D, no statistical significance was observed among the means (Table 1). Φ and ψ changes due to temperature arising were approved in these cases, as confirmed by Ramachandran plots (Figures 3 and 4).

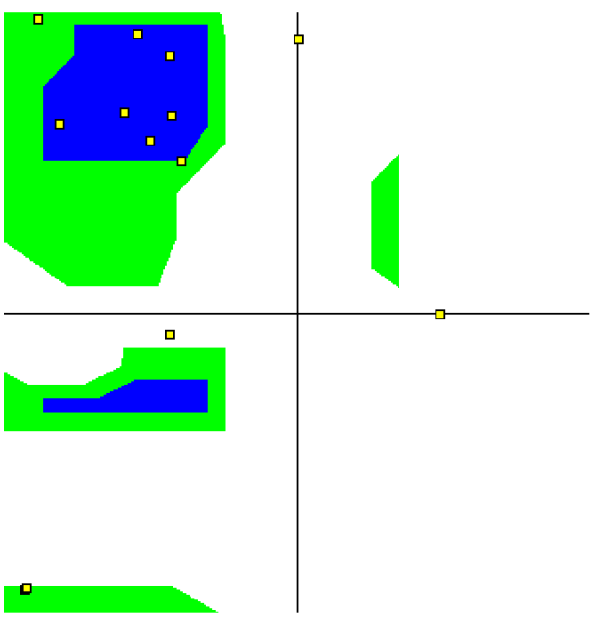
C- Polypeptide had a better situation in water at 310K. At both room temperature and 315K, the tertiary structure of the protein did not show critical changes (Table 2). Similar trend was observed for D- polypeptide chain (Table 3). The behavior of C and D polypeptide chain are are different with respect to the radius of gyration (Rg). The radius of gyration is a factor that describes the dimensions of a polymer chain. It is defined as:

$$R_g^2 \stackrel{\text{def}}{=} \frac{1}{2N^2} \sum_{i,j} (r_i - r_j)^2$$

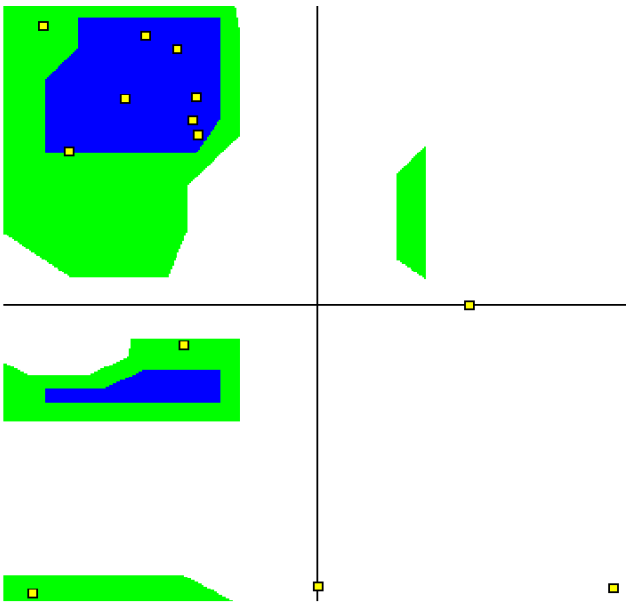
where r_{mean} is the mean position of the monomers. The Rg is proportional to the root mean square distance between the monomers:

$$R_g^2 \stackrel{\text{def}}{=} \frac{1}{N} \sum_{k=1}^{n} (r_{k-} r_{mean})^2$$

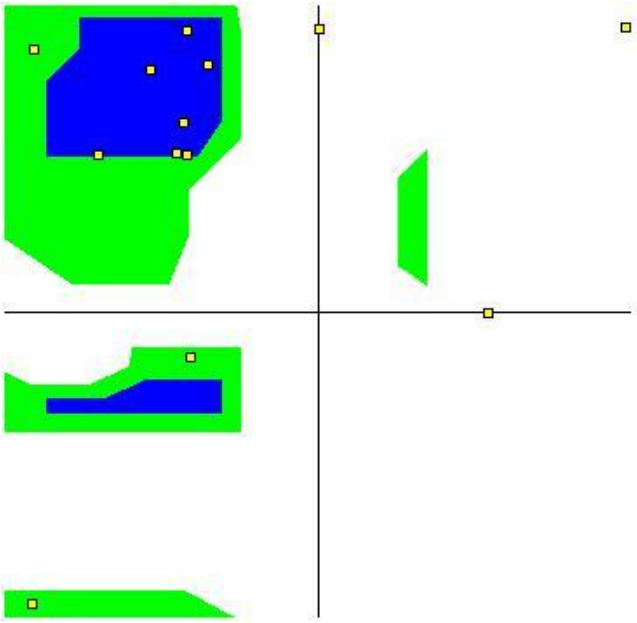
The chain conformations of a polypeptide are quasi infinite in number and constantly change over time. The radius of gyration in polypeptide must usually be



C- Polypeptide at T=298.15K



C- Polypeptide at T=310K



C- Polypeptide at T=315K

Figure 3. Ramachandran plot of C- polypeptide of NS3/NS4 (PDB ID, 1NS3). Changes of critical φ and ψ angels due to Montecarlo simulation in different temperatures are illustrated in plots. This polypeptide is simulated in water medium.

understood as a mean over all polymer molecules of the sample and over time.

$$R_g^2 \stackrel{\text{def}}{=} \frac{1}{N}\left[\sum_{k=1}^{N}(r_k - r_{mean})^2\right]$$

where the brackets define the average.

C- Polypeptide at 310K shows a decrease in Rg, but it increases at 315K again. The Rg of 315K is less than that of 29815K. Temperature had another effect on D chain. It causes the D chain to increase the Rg at 310K and decrease it at 315K. These changes are very small (Table 4). The processes of C and D polypeptides are different when they are substituted in core ring, *Cyclo* [(-

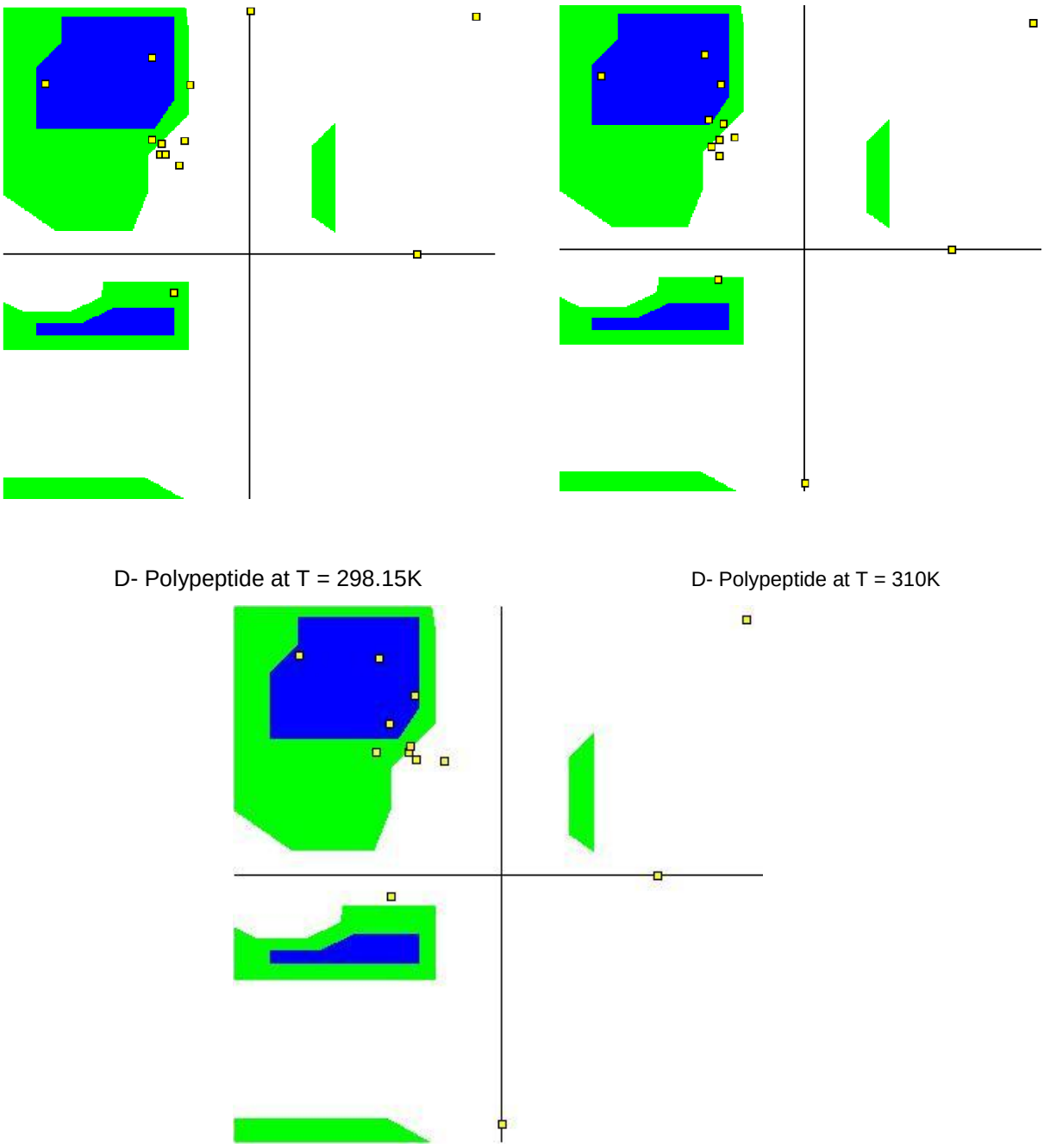


Figure 4. Ramachandran plot of D- polypeptide of NS3/NS4 (PDB ID, 1NS3), illustrated the changed of critical φ and ψ angles due to Montecarlo simulation in different temperatures. The polypeptide is surrounded with water molecules during simulation.

D-G_3-L-D_3)]. Since the trend of the C-chain energy differences in nanostructure was similar to that one separately, it could be concluded that the potential energy changes of this nanostructure was probably a function of C- polypeptide chain changes. But the D-polypeptide chain in combination to core shows another pattern in comparison to D- chain (Figure 5). It is suggested that the pattern was not affected by D- poly peptide individually, and the new nanostructure had its own behavior due to core ring energy changes or the increase of the number of C-poly peptide chains added to a nanoring would cause the mean potential energy to grow. The mean changes of energy in D-chain substituted *Cyclo* [(-D-G_3-L-D_3)] is different. At both 310 and 315K, the nanosystem with two added D-polypeptide showed a larger instability in comparison to 1 and 3

Table 2. Data of φ and ψ angles changes in 13 residues of C- polypeptide.

S/N	Residue	Crystallographic polypeptide			298.15K			310K			315K		
		φ	ψ	Zone	φ	ψ	Zone	φ	ψ	Zone	φ	ψ	Zone
1	LYS	0.00	154.36	W	0.00	164.57	W	0.00	-170.79	W	0.00	166.78	W
2	GLY	-175.56	172.58	G	-71.42	83.67	W	170.56	-171.79	W	176.21	167.90	W
3	SER	-83.47	161.58	B	-98.59	167.25	B	-98.37	162.93	B	-75.55	165.91	B
4	VAL	-77.36	118.25	B	-77.24	118.97	B	-69.18	125.44	B	-77.68	111.17	B
5	VAL	-99.30	139.80	B	-106.76	120.94	B	-110.31	124.13	B	-96.50	142.27	B
6	ILE	-78.36	92.66	B&G	-71.62	91.41	B&G	-68.02	102.89	B	-81.84	93.51	B
7	VAL	-75.68	-13.33	W	-78.89	-12.66	W	-76.19	-24.64	G	-73.99	-26.02	G
8	GLY	-175.48	-177.18	G	-166.86	-164.94	G	-163.97	-174.49	G	-164.71	-171.41	G
9	ARG	-154.54	159.58	G	-146.85	113.85	G	-157.13	168.48	G	-163.85	154.18	G
10	ILE	-129.83	99.71	B	-146.85	113.85	B	-142.37	92.55	B&G	-126.25	93.00	B&G
11	ILE	-72.55	108.44	B	-90.47	103.85	B	-71.29	111.19	B	-75.42	92.86	B&G
12	LEU	-75.32	158.48	B	-78.77	154.46	B	-80.86	154.88	B	-63.30	145.59	B
13	SER	77.99	0.00	W	87.43	0.00	W	87.48	0.00	W	97.07	0.00	W

W, White zone, not allowed zone; G, green, semi allowed zone; B, blue, allowed zone.

Table 3. Data of φ and ψ angles changes in 13 residues of D- polypeptide.

S/N	Residue	Crystallographic polypeptide			298.15K			310K			315K		
		φ	ψ	Zone	φ	ψ	Zone	φ	ψ	Zone	φ	ψ	Zone
1	LYS	0.00	164.78	W	0.00	177.30	W	0.00	-172.76	W	0.00	-167.06	W
2	GLY	-61.47	90.27	G	-71.42	83.67	G	-70.66	95.10	B	-75.35	101.90	B
3	SER	-44.52	119.63	G&W	-43.01	123.44	G&W	-61.83	121.80	B	-58.75	120.14	B
4	VAL	-59.73	80.07	W	-51.32	64.20	W	-62.25	68.92	W	-57.19	77.16	W
5	VAL	-138.39	146.27	B	-71.20	143.67	B	-73.73	143.41	B	-82.52	145.24	B
6	ILE	-72.81	76.65	G&W	-65.86	72.76	W	-62.92	80.28	W	-62.20	82.39	G&W
7	VAL	-55.31	-36.42	G	-55.65	-28.57	G	-63.88	-22.86	G	-74.89	-14.34	W
8	GLY	178.24	169.87	W	165.88	173.89	W	168.41	166.81	W	165.43	171.27	W
9	ARG	-141.29	135.71	B	-149.36	124.96	B	-149.89	127.12	B	-136.08	147.40	B
10	ILE	-71.28	89.33	G	-64.34	80.69	G&W	-59.36	92.68	G	-84.01	82.87	G
11	ILE	-55.90	79.85	W	-47.25	82.98	W	-68.54	75.78	G&W	-38.39	76.15	W
12	LEU	-62.62	78.06	W	-61.54	72.27	W	-51.66	82.34	W	-61.48	86.35	G&W
13	SER	125.39	0.00	W	122.11	0.00	W	0.00	-172.76	W	105.62	0.00	W

W, white zone, not allowed zone; G, green, semi allowed zone; B, blue, allowed zone.

Table 4. Radius of gyration changes of both C and D polypeptides with temperature in water medium.

Parameter	Temperature (K)		
	298.15	310	315
Rg/ C polypeptide	11.6539	11.6515	11.6195
Rg/ D polypeptide	11.0462	11.0574	11.052

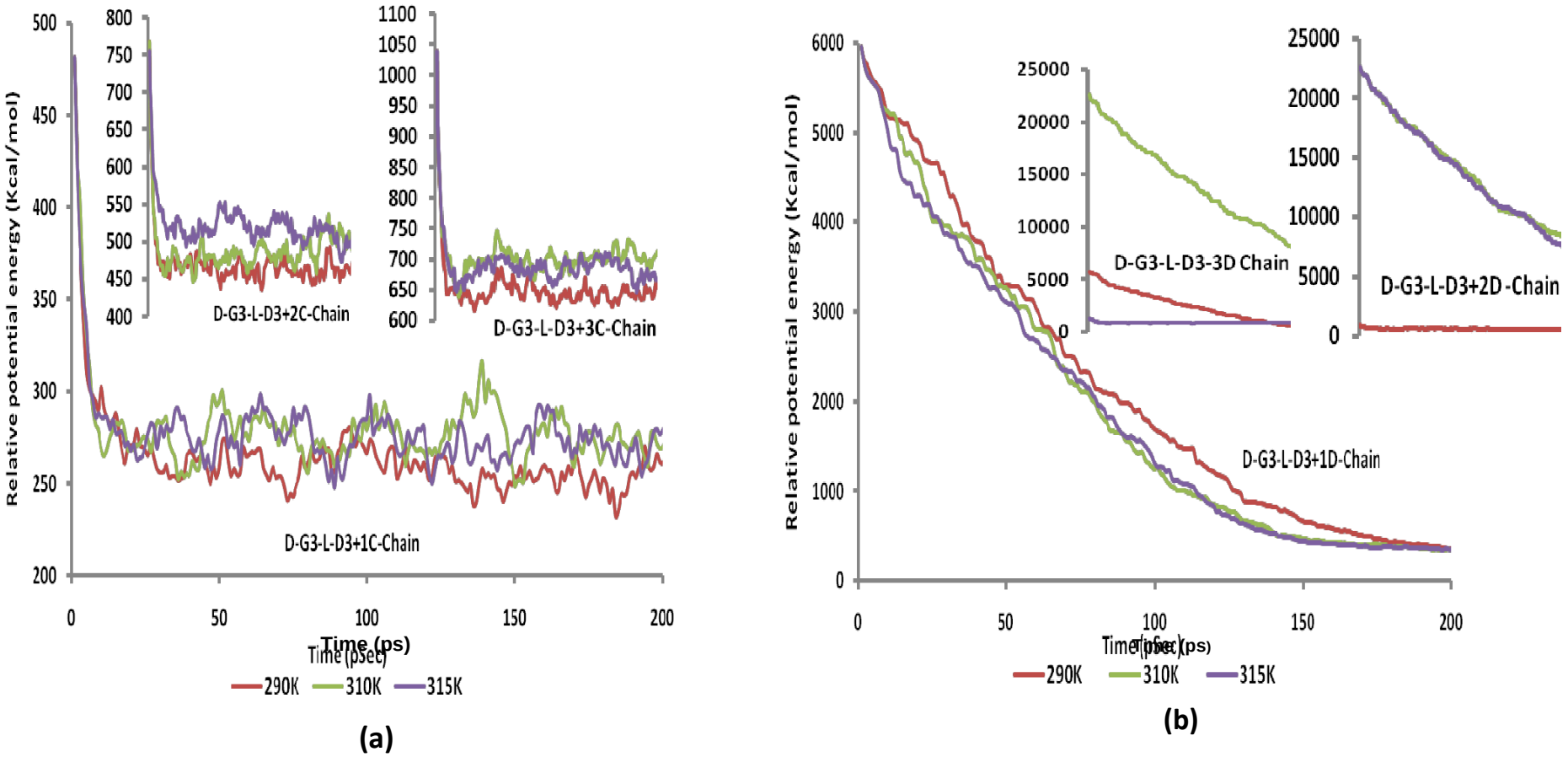


Figure 5. The effect of three critical temperatures of (room, normal body and fever) on nanovaccin structures after Monetecarlo simulation in 200 ps. (a) The relative potential energy changes are demonstrated for three different forms of 1C, 2C and 3C; (b) 1D, 2D and 3D polypeptides in combination to cyclohe xapeptide nanoring.

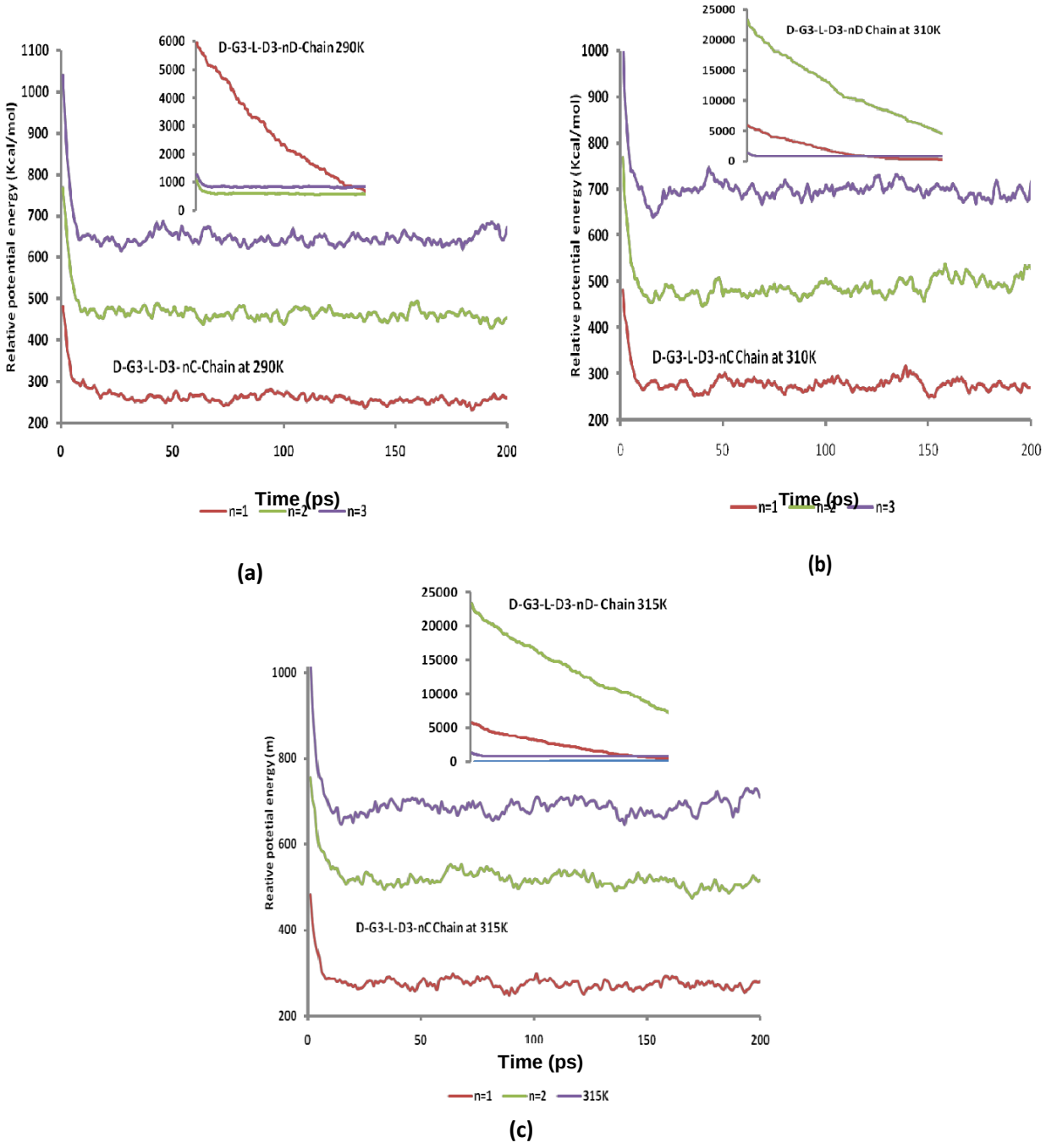


Figure 6. Potential energy changes of nanostructures in 200 ps, after molecular mechanic simulation. Diagrams illustrate the role of increasing the extracted C and D polypeptide to cyclo hexa peptide nanocarrier. Three different temperatures of 290, 310 and 315 were considered to affect on biosystems.

added ones. But it is different at 290K, where 1 chain was added (Figure 6).

DISCUSSION AND CONCLUSIONS

Development of a hepatitis C vaccine is a challenge (Randal, 1999). No effective vaccine is currently available in drug market. Several vaccines are currently under development (Strickland et al., 2008). Hepatitis B core antigen is used as an alternative to challenge (Chen and Li, 2006). Non structural protein, NS3 can stimulate TH1 and make enhancement in NS3-specific interferon-γ (IFN-γ) amount. To find a novel approach to deliver epitops as vaccine, it is thought to use nano-particles which are biodegradable and are not harmful to

body.

Adding the consensus polypeptides substituent such as NS3 components on cyclic hexa peptide nanorings with the ability to stimulate immune system without any risk of infection is the main goal of this vaccine design. Since the peptide nanoring has the potential of self assembly, it is thought that after making nanovaccine based on nanocyclohexa peptides, it will make a larger structure such as a tube. Although both C and D polypeptide chains are stable in water medium such as body condition, the results revealed that D chain is more stable. Since higher temperature (fever) can be critical in protein denaturation and it is an important symptom in infections, a greater stability of a nanovaccine in this condition could be very important. Based on our simulations, the *Cyclo* [(-D-G_3-L-D_3)] with three substituent of D-chain is suggested for a nanovaccine purpose.

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