Activation of human insulin by vitamin E: A molecular dynamics simulation study

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Abstract:

Lack of perfect insulin signaling can lead to the insulin resistance, which is the hallmark of diabetes mellitus. Activation of insulin and its binding to the receptor for signaling process initiates via B-chain C-terminal hinge conformational change through an open structure to “wide-open” conformation. Observational studies and basic scientific evidence suggest that vitamin D and E directly and/or indirectly prevent diabetes through improving glucose secretion and tolerance, activating calcium dependent endopeptidases and thus improving insulin exocytosis, antioxidant effect and reducing insulin resistance. On the contrary, clinical trials have yielded inconsistent results about the efficacy of vitamin D supplementations for the control of glucose hemostasis. In this work, best binding modes of vitamin D$_3$ and E on insulin obtained from AutoDock Vina were selected for Molecular Dynamic, MD, study. The binding energy obtained from Molecular Mechanics- Poisson Boltzman Surface Area, MM-PBSA method, revealed that Vitamins D$_3$ and E have good affinity to bind to the insulin and vitamin E has higher binding energy (-46 kj/mol) by engaging more residues in binding site. Distance and angle calculation results illustrated that vitamin E changes the B-chain conformation and it causes the formation of wide-open/active form of insulin. Vitamin E increases the Val$^{B12}$-Tyr$^{B26}$ distance to ~15 Å and changes the hinge angle to ~65°. Consequently, essential hydrophobic residues for binding to insulin receptor exposed to surface in the presence of vitamin E. However, our data illustrated that vitamin D$_3$ cannot change B-chain conformation. Thus our MD simulations propose a model for insulin activation through vitamin E interaction for therapeutic approaches.

Keywords: Insulin, Hinge opening, Diabetes mellitus, Molecular dynamics simulation, MM-PBSA
Abbreviations:

COM, Center Of Mass; LINCS, Linear Constraint; MD, Molecular Dynamics; MM-PBSA, Molecular Mechanics- Poisson Boltzman Surface Area; PDB, Protein Data Bank; SPC, Simple Point Charge; PME, Particle Mesh Ewald; R_g, Gyration Radius; RMSD, Root Mean Square Deviation; SPC water, Simple Point-charge Water; VMD, Visual Molecular Dynamics;

Introduction:

Observational studies and basic scientific evidence suggest that vitamin D and E directly and/or indirectly prevent diabetes through improving glucose secretion and tolerance, activating calcium dependent endopeptidases and thus improving insulin exocytosis, antioxidant effect and reducing insulin resistance [1-3]. It has been reported that type 2 diabetes mellitus patients are associated with vitamin D_3 deficiency [4]. Recently, it has been demonstrated that vitamin D supplementations are inversely affiliated with insulin resistance, which may have beneficial effects on controlling several complications of childhood obesity [5]. Vitamin D and E supplementations could diminish insulin resistance in patients with non-insulin dependent diabetes mellitus with substantial improvements in serum fasting plasma glucose (FPG) and insulin [6, 7]. New clinical trial on 2,423 participants has been established that vitamin D supplementations decrease the risk of diabetes [8]. F. Cadario et. al in 2017 reported that co-administration of omega 3 fatty acid and high dose vitamin D could assist to halt the progression of type 1 diabetes [9]. Low dairy intake of vitamin E supplementations during the second pregnancy trimester can dwindle the risk of hyperglycemia and insulin resistance [10].
Supernumerary evidence has strongly proposed that vitamin D plays a significant role in amending the risk of diabetes. However, the findings of type 2 diabetes are derived particularly from observational and basic studies, which may be missed by a variety of parameters. Only a few and often underpowered clinical trials have been designed to test the effectiveness of vitamin D and E administration in diabetes [11]. Many ongoing randomized trials are trying to test the hypothesis that vitamin D and E lower type 2 diabetes risk [12].

Insulin is a peptide produced by beta cells of islets of pancreas that plays its role as one of the main anabolic hormones in the body. It regulates the metabolism of proteins, fats, and carbohydrates by promulgating the absorption of glucose from the blood into skeletal muscle, fat and liver cells [13]. Investigations in the last century illustrated that insulin served a key role in the advancement of structural biology, peptide chemistry, pharmacology and cell signaling [14, 15].

The functional and biologically active form of insulin is a monomer comprising 51 amino acids containing two chains, an A-chain of 21 and a B-chain of 30 residues, which are linked together by two inter-chain disulfide bridges between Cys\textsuperscript{A7}-Cys\textsuperscript{B7} and Cys\textsuperscript{A20}-Cys\textsuperscript{B19}. The A-chain has an intra-chain disulfide bridge between Cys\textsuperscript{A6}-Cys\textsuperscript{A11} as well. The A-chain has an N-terminal helix from Gly\textsuperscript{A1} to Thr\textsuperscript{A8}, continued with an antiparallel C-terminal helix from Ser\textsuperscript{A12} to Cys\textsuperscript{A20}. The B-chain also has two N- and C-terminal strands surrounding a central helix Gly\textsuperscript{B8} to Cys\textsuperscript{B19}. This conformation is known as T conformation (Fig. 1a and b). At micromolar concentration, insulin dimerizes through a $\beta$ sheet structure of both C-terminal portions of the B-chain, and in the higher concentrations in the presence of zinc ions associates into the beautiful symmetrical structures of hexamer [16, 17].
Fig. 1. **Structural details of the insulin molecule and vitamins D₃ and E.** (a) 3D structure of human insulin (PDB ID: 2G4M). A-chain (yellow); B-chain N-terminal (residues Phe⁸ to Gly⁸) (red), B-chain α-helix (residues Ser⁹ to Cys¹⁹) (black), B-chain C-terminal (residues Gly²⁰ to Thr³⁰) (blue). The colored spheres demonstrate the Cα atoms of the first and last residues of the A and B-chains. (b) Side chains of the insulin hydrophobic core: Gly⁸, Leu¹¹, Val¹², Leu¹⁵, Phe²⁴, Tyr²⁶ (shown in purple), Ile² and Val³ (shown in green).

The mechanism of monomeric insulin conformational changes and its engagements with the receptor has long been still enigmatic [18]. Attention has been recently focused on B-chain C-terminus portion that is critical for self-assembly in β cells and receptor attachment in target tissues. In particular, the C-terminal residues of the B-chain (nearly Phe²⁴-Thr³⁰) egress from the helical core to divulge buried nonpolar surfaces (the detachment model) [19]. Therefore, the C-terminal β-strand (Phe²⁴-Thr²⁷) and β-turn (Gly²⁰-Gly²³) rotate away from the α-helical core of insulin to expose its conserved aromatic motif (Phe²⁴, Phe²⁵, Tyr²⁶) to insulin receptor binding site [20]. Most recent findings showed that opening of B-chain C-terminus is inherently stochastic and then transition through an open structure to “wide-open” conformation. The wide-open conformation is critical for receptor binding, however, this conformation occurs very seldom. B-chain C-terminus opens as a zipper mechanism; also Phe²⁴ acts as a hinge that is retained in the prevailing closed/inactive state by neighboring Tyr²⁶ hydrophobic effect. The Tyr²⁶ is the essential residue for initiation of B-chain C-terminal opening and activation of insulin [21].
Engagement of insulin to its receptor in the primary hormone binding site (α-subunit domains L1 and α-CT) occurs due to hinge-like rotation at insulin’s Gly$^{B20}$-Gly$^{B23}$ β-turn, coupling reorientation of Phe$^{B24}$ to a 60° rotation of the Phe$^{B25}$-Thr$^{B30}$ away from the protein hydrophobic core. Inauguration of this hinge exposes conserved nonpolar side chains (Phe$^{B24}$, Phe$^{B25}$, Val$^{B12}$, Ile$^{A2}$ and Val$^{A3}$) to engage the receptor [22]. Restraining the hinge reconstitution by nonstandard mutagenesis preserves native conformation but blocks receptor binding [20, 23].

Different strategies have been reported to treat diabetes, such as immunomodulation [24], metabolic/bariatric surgery [25, 26], islet transplantation [27], amino acid substitution for insulin stability improvement [28] and ligand binding [29, 30]. In the last decade, vitamins have scintillated more attention to diabetes treatment [31]. Vitamin D directly and/or indirectly prevents diabetes through improving glucose secretion and tolerance, activating calcium dependent endopeptidases and thus improving insulin exocytosis, antioxidant effect and reducing insulin resistance [2, 32].

Phenolic components widely used for maintaining insulin preparations can have a profound result on the insulin’s conformation in solution [33]. Phenol group stabilizes more helix structures in the insulin hexamer [34]. This conformational change can affect the hormone’s stability and function [35]. Vitamin E as a phenolic compound reduces oxidative stress and protein glycosylation as well as improves insulin sensitivity and insulin action after oral administration [7, 10]. Recently, it was found that cholecalciferol and α-tocopherol -the active forms of vitamin D and E in blood stream in the vicinity of insulin, strongly bind to hydrophobic patch of human insulin and change insulin’s structure and improve its stability [36].

In the present work, we investigated the interactions between α-tocopherol and cholecalciferol with human insulin monomer individually using molecular dynamic
simulation approach. The aim was to study the effect of vitamin binding on insulin B-chain conformational transition, which play an important role in the insulin/insulin receptor attachment as a critical component for insulin function.

2. Methods:

2.1. Molecular docking

In the MD simulations, we used a crystal structure of human insulin monomer with a resolution of 1.35 Å (PDB ID: 5ENA [37]). Vitamins structures were obtained from the Brookhaven Protein Data Bank (PDB IDs: 3C6G [38] for vitamin D$_3$ and 3CXI [39] for vitamin E). Molecular docking was done using AutoDock Vina with standard parameters [40]. Both vitamins were docked to the human insulin monomer in $26 \times 26 \times 34$ Å grid box. Best binding structures for each vitamin were selected using the best score of AutoDock Vina results. By analyzing the binding energy and RMSD, certain insulin-vitamin complexes were selected for simulation studies.

2.2. MD simulations

MD simulations were performed using the Gromacs 4.6.7 package using Gromos96 53a6 force field [41, 42]. The ligands topologies were generated by the PRODRG server [43]. The structure of insulin monomer was solvated in SPC water molecules. Na$^+$ and Cl$^-$ ions were placed in the solvated system to neutralize it and to set the NaCl concentration to 0.1 M. The system was then exposed to a primary minimization of water molecules followed by equilibration. Steepest descent energy minimization was performed down to a maximum gradient of 1000 KJmol$^{-1}$nm$^{-1}$. The system was simulated in two steps, 1000 ps of position restrained dynamics using LINCS algorithm followed by full 100 ns standard MD [44]. The system was coupled to a Berenson’s thermostat method with the reference temperature of 300
k, further, standard pressure of 1.0 bar with a coupling constant of 1000 ps for pressure. It is noteworthy, during this time, positions of all bonds were constrained. PME electrostatics were applied using the Lennard-Jones cutoff of 1.4 nm and a Coulomb cutoff of 0.9 nm [45]. MD simulations were done during 100 ns for all minimized structures with time step of 2 fs. Equilibrium of the system was monitored from the RMSD plot. Images were created using PyMOL molecular graphic system [46], VMD [47] and the program LigPlot+ v 1.0 [48], which generates schematic 2-D representations of protein ligand complexes from the PDB file input [49, 50].

2.3. MM-PBSA analysis

The binding affinity of vitamins could be investigated on the basis of binding free energy. Gibbs free energy was calculated by the procedure MM-PBSA [51, 52]. Interaction energy for insulin with vitamin D₃ and E were calculated. MM-PBSA methods were implemented for 90 to 100 ns. For each simulated system, 1000 snapshots were taken from the last 10 ns of the trajectory at the intervals of 10 ps. In this study, different components of interaction energy which contribute to vitamin binding were estimated. This includes van der Waals interactions, electrostatic interactions, polar and non-polar solvation energy. The MM-PBSA analysis also offers a unique contribution to the binding affinity and per residue distribution that provides significant amino acid in all cases. Energy decomposition per residues and MM-PBSA revealed the relative importance of amino acid involved in binding and different components of binding energy.
3. Result and Discussion:

3.1. Molecular docking

The blind-docking results indicated that insulin monomer has only one binding site for vitamins. The grid dimensions for accurate-dock were adjusted according to the binding site. AutoDock Vina scored twenty interaction modes for each complex based on the affinity and distance from the best mode. Best docking conformations of vitamin-insulin complexes, generated by Autodock, were taken as initial structure for MD simulation. Table 1 shows the binding energies of the chosen complexes. Vitamin D$_3$ and E bind to hydrophobic groove of insulin with the lowest binding energy of -29.7 and -36.5 kJ/mol, respectively.

Interaction modes and the orientation of vitamins are also illustrated in Fig. 2. In Fig. 2, it is shown both vitamins D$_3$ and E are bound to hydrophobic region between two $\alpha$-helix structures in A and B-chain interface. After binding of vitamins, the B-chain C-terminal hinge is free to couple with its receptor by formation of wide-open conformation. In other words, vitamins do not inhibit insulin interaction with its receptor and insulin activity is maintained. Fig. 3 shows the hydrophobic interactions between insulin and vitamins created by LigPlot$^+$ program in 2D representation [53]. Leu$^{A13}$, Tyr$^{A14}$, Glu$^{A17}$, Phe$^{B1}$, Gln$^{B4}$, Ala$^{B14}$, Leu$^{B17}$, Val$^{B18}$ and Arg$^{B22}$ residues participate in hydrophobic interaction.

Table 1. Binding energy. Obtained binding energies of the molecular docking and MM-PBSA method for vitamin D$_3$ and vitamin E.

<table>
<thead>
<tr>
<th></th>
<th>Insulin-Vitamin D$_3$</th>
<th>Insulin-Vitamin E</th>
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<tr>
<td>Docking Binding Energy (kJ/mol)</td>
<td>-29.7</td>
<td>-26.8</td>
</tr>
<tr>
<td>MD Binding Energy (kJ/mol)</td>
<td>-36.5</td>
<td>-46</td>
</tr>
</tbody>
</table>
Fig. 2. The orientations of the $\text{D}_3$ (shown in blue) (a) and vitamins E (shown in green) (b) bound to the A-chain; Hydrophobic surface schematics of human insulin monomer interacted with vitamin $\text{D}_3$ (c) and vitamin E (d). Insulin is shown in hydrophobic representation.

Fig. 3. 2D plot of protein (insulin)-ligand (vitamin $\text{D}_3$ and E) interaction (generated by LigPlot' obtained from docking results). Ligplot of insulin with vitamin $\text{D}_3$ (a). Ligplot of insulin with vitamin E (b). Red semicircle shows hydrophobic interactions.
3.2. Conformational stability of insulin

MD simulation is a reliable method to find out the structural movement of molecules and atoms as a function of time. To explore the conformational stability of insulin and effective conformation samplings during the MD simulations, the root mean square deviation (RMSD) was computed based on the starting structure. The RMSD method averaging all the particles at a time. This action is based-on the comparison of the structure after the simulation with the first structure and the smaller its numerical value, the simulated system is more stable [54].

The backbone RMSD of A and B-chain and \( R_g \) (gyration radius) of insulin-vitamin complexes in comparison to the apo-insulin are plotted in Fig. 4 and average values are shown in Table 2. The entire conformation of the apo-insulin structure was observed stable about 0.12 and 0.3 nm for A and B-chain, respectively, during the simulation. The RMSD was generally stable, however, some interesting variations occurred in B-chain insulin, which is related to B-chain C-terminal fluctuation. In the insulin-Vitamin E simulation, the average RMSD of B-chain was calculated about 0.5 nm (Fig. 4b). As expected, the RMSD of insulin-Vitamin E shows persistent variation, probably because of phenol effect on the insulin conformational changes and hinge opening [55].

The radius of gyration (\( R_g \)) was measured to examine the dynamic stability and compactness of protein in the presence of vitamins. A time evolution plot of \( R_g \) (backbone) is shown in Fig. 4c. Several consecutive and continuous fluctuation is seen in \( R_g \) trajectory from beginning of simulation, which is due to major structural changes of protein. This drift in \( R_g \) is maintained up to 50 ns. Thereafter, a constant fluctuation is seen up to 75 ns,a gain in \( R_g \) is observed up 90 ns. The vitamin E changes the gyration radius of insulin due to its influence on the B-chain hinge conformational transition.
Fig. 4. **RMSD and Gyration radius.** RMSD of backbone atoms of insulin-vitamin complexes in respect to apo-insulin for A-chain (a) and B-chain (b). $R_g$ of whole protein with respect to 100 ns molecular dynamics simulations (c).

Table 2. RMSD profile of protein backbone atoms and $R_g$ profile of whole protein calculated over the course of 100 ns molecular dynamic simulations.

<table>
<thead>
<tr>
<th></th>
<th>Apo-Insulin</th>
<th>Insulin-Vitamin D$_3$</th>
<th>Insulin-Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A-chain RMSD (nm)</strong></td>
<td>0.12</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>B-chain RMSD (nm)</strong></td>
<td>0.3</td>
<td>0.27</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>$R_g$ (nm)</strong></td>
<td>1.03</td>
<td>1.02</td>
<td>1.05</td>
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</table>

### 3.3. MM-PBSA analysis

To estimate the strength of interaction between vitamins and insulin and difference in the binding energy between vitamins, MM-PBSA method was applied for 98 to 100 ns. The obtained values indicated that the contribution of van der Waals interaction in the total interaction energy was much larger than the electrostatic energy (Table 3). The positive value of polar solvation energy indicated a little contribution to vitamin binding with insulin [56]. In order to recognize the hotspot residues of insulin ligand binding, the contribution of every residue was explored. Per residue binding energy decomposition for insulin with both vitamins suggested that $\text{Ile}^{\text{A10}}, \text{Leu}^{\text{A13}}, \text{Leu}^{\text{A16}}, \text{Phe}^{\text{B1}}, \text{Gln}^{\text{B4}}, \text{Leu}^{\text{B6}}, \text{Ala}^{\text{B14}}, \text{Leu}^{\text{B17}}, \text{Val}^{\text{B18}}$ were the major residues interacting with vitamins (Fig. 5). Per residue energy contributions
illustrated that the favorable binding free energy originates predominantly from the nonpolar terms.

Table 3. Binding energy values (kJ/mol) and individual component energy calculated with MM-PBSA method for insulin with vitamin D$_3$ and vitamin E.

<table>
<thead>
<tr>
<th></th>
<th>Van der Waal energy</th>
<th>Electrostatic energy</th>
<th>Polar solvation energy</th>
<th>SASA energy</th>
<th>Binding energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-Vitamin D$_3$</td>
<td>-40.413 +/- 5</td>
<td>-0.258 +/- 0.18</td>
<td>9.453 +/- 2.12</td>
<td>-5.233 +/- 0.65</td>
<td>-36.451 +/- 4.14</td>
</tr>
<tr>
<td>Insulin-Vitamin E</td>
<td>-54.087 +/- 7.1</td>
<td>-0.079 +/- 0.085</td>
<td>14.281 +/- 2.67</td>
<td>-6.099 +/- 0.799</td>
<td>-45.984 +/- 5.41</td>
</tr>
</tbody>
</table>

Fig. 5. Energy decomposition graph for individual amino acid residues majorly contributing to binding energy. Ile$^{A10}$, Leu$^{A13}$, Leu$^{A16}$, Phe$^{B1}$, Gln$^{B4}$, Leu$^{B6}$, Ala$^{B14}$, Leu$^{B17}$, Val$^{B18}$ were the major residues interacting with vitamin E.

3.4. Opening of B-chain C-terminal hinge

There are several strategies for investigation of B-chain C-terminal conformational changes to study how vitamins can affect insulin structure and function. Distance computation between B-chain C-terminal and $\alpha$-helix residues and also calculating angle changes of B-chain used for hinge conformational transition analysis. We selected three pair residues from
the B-chain C-terminal and B-chain α-helix for calculating distances of pair residues and studying conformational changes of B-chain. Gly$_{B8}$, Val$_{B12}^{B}$ and Leu$_{B15}^{B}$ were selected from α-helix portion and distances were calculated from 3 C-terminal residues (Phe$_{B24}^{B}$, Tyr$_{B26}^{B}$, Pro$_{B28}^{B}$) for all trajectories. Fig. 6a shows the distances between the C$_α$ atoms of the three pairs from the MD simulation. These distances provide the most useful indexes for opening of the B-chain C-terminus portion and hence must pay more attention to it.
Fig. 6. **Illustration of the residue pairs** for (a) distance calculation and (b) Angle computation. The spheres represent the Cα atoms of the B-chain α-helix and B-chain C-terminal residues, respectively. (c and e) Up and lateral view of Insulin-Vitamin E complex, and (d and f) Up and lateral view of Insulin-Vitamin D₃ complex after simulation.
The MD simulation result for the vitamin apo-insulin shows that it mainly remains in the closed conformation and the B-chain C-terminus portion makes only sporadic excursions away from the B-chain $\alpha$-helix (Fig. 7). Fig. 7 shows the stability of hinge in apo-insulin. Distance calculation in apo-insulin hinge shows distance in all indexes remain under 8.5 Å. Based on previous experimental and computational results, calculated distance in apo-insulin B-chain hinge is about 10 Å, so B-chain hinge is in the closed conformation. These findings were validated by comparing with the recently reported MD simulations by A. Papaioannou, et al. [21] and corresponding crystal structure 2G4M. The average distances between the C$_\alpha$ atoms of B-chain C-terminal and B-chain $\alpha$-helix approximate with the experimental values within 10 Å (Fig. 7). As displayed in Fig. 1b, the side chains of Phe$^{B24}$, Phe$^{B24}$ and Tyr$^{B26}$ participate in the formation of hydrophobic core, which play the main role of maintaining insulin in the closed state.

Gly$^{B8}$-Pro$^{B28}$ and Val$^{B12}$-Tyr$^{B26}$ distances provide the most useful indicators for opening and wide-opening of the B-chain C-terminal, respectively. There is a good confirmation between our results and the experiments, which further validates the MD simulations. Obtained results show the Leu$^{B15}$-Phe$^{B24}$ distance of apo-insulin does not change, in accordance with a hinge behavior, while others increase to varying values. Average distances between the C$_\alpha$ atoms of the pairs Leu$^{B15}$-Phe$^{B24}$, Val$^{B12}$-Tyr$^{B26}$ and Pro$^{B28}$-Gly$^{B8}$ are approximately 7.5, 8.3 and 6.8 Å respectively. It is obvious from all the MD simulations that there is no systematic dynamic behavior or opening mechanism like a switch or periodic motion between two states. The insulin activation casually occurs at the simulated times and appears to be an accidental event (Fig. 7).
Fig 7. Stability of the hinge. Distances between the Cα atoms of the LeuB15-PheB24, ValB12-TyrB26 and GlyB8-ProB28 pairs, which represent at the hinge, for the apo-insulin.

Time series of the distances between Cα atoms of GlyB8-ProB28 illustrated that open conformation of B-chain C-terminal hinge is formed affected by vitamin E (Fig. 8a). As it is obvious from the time series of the distances between Cα atoms of ValB12-TyrB26, increasing of distance and opening of hinge occurred only in the presence of vitamin E and hence only vitamin E can induce the wide-open conformation, not vitamin D3 (Fig. 8b). ValB12-TyrB26 average distance increased from 0.83 Å in apo-insulin to 1.34 Å in insulin-Vitamin E that clearly shows the wide-open conformation.
Fig. 8. **Time series of the distances between the C\(_\alpha\) atoms** of (a) Gly\(^{B8}\)-Pro\(^{B28}\), (b) Val\(^{B12}\)-Tyr\(^{B26}\) and (c) Leu\(^{B15}\)-Phe\(^{B24}\). These distances are used as a criterion for the opening of the B-chain hinge and activation of insulin.

Examination of the time series of the distances of insulin hinge in insulin-Vitamin D\(_3\) complex shows that the Val\(^{B12}\)-Phe\(^{B24}\) and Gly\(^{B8}\)-Pro\(^{B28}\) distances do not change, so vitamin D\(_3\) could not activate insulin for binding to its receptor. Distances between C\(_\alpha\) atoms of Leu\(^{B15}\)-Phe\(^{B24}\) shows B-chain hinge most stable in all simulations, illustrated the importance of \(\beta\)-turn in insulin structure.

How insulin changes conformation to engage the insulin receptor has long been unclear. The recent structural experiment of insulin and its receptor demonstrated that B-chain protective hinge of insulin opens to initiate receptor binding procedure [20]. It explained that, on receptor binding, B-chain C-terminal fragment undergoes reorientation of Phe\(^{B24}\) to a 60° rotation of the Phe\(^{B25}\)-Lys\(^{B29}\) strand away from the insulin core, to expose buried residues to its receptor.

The angle changes were calculated for the confirmation of distance results and characterization of the wide-open conformation. To analysis of angle changes in B-chain C-terminal, Gly\(^{B8}\), Glu\(^{B21}\) and Lys\(^{B29}\) were selected for structure of open conformation (Phe\(^{B25}\)-Lys\(^{B29}\)). Since wide-open conformation occurs in the B-chain hinge (Phe\(^{B24}\)-Tyr\(^{B26}\)), Gly\(^{B8}\), Glu\(^{B21}\) and Tyr\(^{B26}\) were selected for the computation of wide-open conformation. In Fig 9(a and b), representative angle variation throughout the trajectories for all simulations are shown; four C\(_\alpha\) atoms were selected for angle calculation. The positions of these atoms provide a good representation of the movement of the C-terminal coil relative to the \(\alpha\)-helix and opening of the hinge: Glu\(^{B21}\) is situated in the center of the hinge, which is between helix and coil structure, Gly\(^{B08}\) is situated at the opposite side of helix in front of the coil structure, Lys\(^{B29}\) is situated at the flexible end of C-terminus coil, and Tyr\(^{B26}\) is situated in the middle of
the C-terminus coil and instantly after β-turn, which is the key residue in formation of the wide-open conformation. Accordingly, Cα atoms of these amino acids move as the hinge opens.

Fig. 9. Variation of the B-chain hinge angle along the MD simulation trajectories. The angles are measured between Cα atoms of GlyB8, GluB21 and (a) LysB29, (b) TyrB26. Hydrogen bond of B-chain hinge with A-chain in (c) apo-insulin and (d) insulin–Vitamin E.

The maximum opening for the apo-insulin in the ValB12-TyrB26 is ~8 Å (Fig. 7), while surprisingly vitamin E increases the ValB12-TyrB26 distances to ~15 Å (Fig. 8b). The average opening for the apo-insulin is ~20 degrees (GlyB8, GluB21 and LysB29) (Fig. 9a) and ~30 degrees (GlyB8, GluB21 and TyrB26) (Fig. 9b), while in the presence of vitamin E, the hinge
opens to ~85 and ~65 degrees, respectively. Reorientation of Phe\textsuperscript{B24} to a 60\textdegree rotation of the Phe\textsuperscript{B25}-Thr\textsuperscript{B30} away from the protein hydrophobic core occurs in wide-open conformation of insulin [20]. Obtained results reveal that vitamin E bounded to A and B-chain interface induces conformational changes in the B-chain hinge and obviously opens it.

A contact map is an exclusively useful 2D representation of aminoacid-aminoacid interactions in protein 3D structure [57]. Intramolecular contact maps were computed as shown in Fig. 10. The contact maps clearly show the contact changes in the presence of vitamins. An inspection of this contact maps reveal that distances of Phe\textsuperscript{B25}-Thr\textsuperscript{B30} from Gln\textsuperscript{B4}-Val\textsuperscript{B18} increases in the insulin-Vitamin E complex related to the apo-insulin. As can be clearly seen from distance and angle variation and contact map results, the wide-opening of the B-chain hinge occurs in the presence of vitamin E. Vitamin D\textsubscript{3} does not affect on the Activation of insulin by hinge opening.

![Fig. 10. Contact map results. Mean smallest distance for insulin in the presence of vitamins. Distances of Phe\textsuperscript{B25}-Thr\textsuperscript{B30} from Gln\textsuperscript{B4}-Val\textsuperscript{B18} increases in the insulin-Vitamin E complex related to the apo-insulin.](image-url)

### 3.5. Secondary structure
Insulin forms a small helix-rich (44%) globular protein with 30% random coil, 9% β-strands and 19% turns [58]. The Dictionary Secondary Structure of Proteins (DSSP) analysis performed to identify the secondary structures of each insulin residue in all MD simulations (Fig. 11). The DSSP analysis showed that the secondary structure elements are intact in the apo-insulin (Fig. 11a). As observed in Fig. 11b, the insulin secondary structures began to lost its helical contents mostly in 3-9 residues at the presence of vitamin D₃, which is in agreement with our previous experimental study [36]. Vitamin D₃ destroys helical structure at Gly⁴¹-Cys⁸ residues, which is somewhat unstable in apo-insulin. The DSSP analysis also revealed that vitamin E does not change the helical contents of human insulin during the MD simulation. As it is shown in Fig. 11, β-bridges (isolated backbone hydrogen bonds) are present in residues Cys⁴¹ and His⁸ of apo-insulin. In insulin-Vitamin D₃ complex Cys⁴¹ and His⁸ β-bridges deform in the last 20 ns of simulation time. In insulin-vitamin E complex Cys⁴¹ and His⁸ β-bridges fully deform and Cys²⁰ and Phe²⁴ β-bridges form, which are stable during MD simulation. Vitamin E also destroys Cys¹⁹-Arg²² turn structure by disrupting hydrogen bonds between Tyr¹⁹-Phe²⁵. It seems that Tyr¹⁹-Phe²⁵ hydrogen bond disruption may change the turn structure and induces hinge conformational changes.
Conclusion:

Vitamin D and E supplementations are conventionally used as a long-term oral intake for diabetes treatment. In this work, for the first time, we investigated the interaction of vitamin E and vitamin D₃ with human insulin by computational insights. Our binding energy MM-PBSA calculation results reveal that Vitamins D₃ and E bind to the insulin, also vitamin E...
engages to more residues and reaches high binding energy of -46 kj/mol. Our data is in agreement with our previous experimental results [36]. Per residue energy contributions illustrated that the favorable binding free energy originates predominantly from the nonpolar terms. Ile$^{A10}$, Leu$^{A13}$, Leu$^{A16}$, Phe$^{B1}$, Gln$^{B4}$, Leu$^{B6}$, Ala$^{B14}$, Leu$^{B17}$, Val$^{B18}$ were the major residues interacting with vitamins. Distance and angle calculation results illustrated that vitamin E changes the B-chain conformation and it causes the formation of wide-open/active form of insulin. Val$^{B12}$-Tyr$^{B26}$ distance provides a useful index for studying the conformational changes and forming wide-open structure of B-chain hinge. Vitamin E increases the Val$^{B12}$-Tyr$^{B26}$ distance from ~8 Å in apo-insulin to ~15 Å. In addition, the hinge angle arises to ~65° in the presence of vitamin E. Distance and angle histogram show that wide-open conformation is most stable in insulin-Vitamin E complex. On the other hand, vitamin D cannot change the hinge conformation, therefore, activation of insulin does not occur in insulin-Vitamin D$_3$ complex. The DSSP analysis revealed that insulin secondary structure does not much change in both vitamin-insulin complexes. It seems that vitamin E increases the helical contents of dimeric insulin, due to its phenolic structure, and it does not lead to significant changes in the monomeric insulin helical contents.

As a result, our MD simulations propose a model for insulin activation through vitamin E interaction for therapeutic approaches. In conclusion, these findings provide new insights on the conformational changes of insulin B-chain hinge that is crucial to enable its binding to the insulin receptor. Finally, we propose a new approach for activation of insulin without any gene manipulation or mutagenesis for diabetes treatment.

Appendix A. Supplementary Data:

MD simulations repeated using the Gromacs 2019.2 package and supplementary appendix has been written as per reviewer’s suggestion.
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References: Uncategorized References


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Highlights:

- Imperfection of insulin signaling can lead to insulin resistance, which is the hallmark of diabetes mellitus. Vitamin E and D₃ play a crucial role in insulin sensitivity improvement and diabetes treatment.

- Activation of insulin and binding to its receptor for signaling process initiates via B-chain C-terminal hinge conformational change.

- The wide-open structure of B-chain hinge is critical for receptor binding. Val12-Tyr26 distances provide the most useful indicator for wide-open conformation.

- Vitamin E effectively activates human insulin monomer via B-chain hinge opening. Val12-Tyr26 distances of apo-insulin increases from ~8.3 Å to ~13 Å and hinge angle increases from ~30° to ~65° in the presence of vitamin E.