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Design of an interface peptide as new inhibitor of human glucose-6-phosphate dehydrogenase

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PDH) is an essential enzyme involved in the first reaction of the oxidative branch of the pentose phosphate pathway (PPP). Recently, G6PDH was suggested as a novel target protein for cancer therapy as one of the final products of the PPP, ribose-5-phosphate, is necessary for nucleic acid synthesis and tumor progression. After analyzing the protein–protein interface of the crystal structure of human G6PDH by means of molecular dynamics simulations, we designed six interface peptides based on the natural sequence of the protein. The three most promising peptides, as predicted by binding free energy calculations, were synthesized and one of them was confirmed as a novel inhibitor of human G6PDH in experimental assays. Together, the active peptide found and its suggested binding mode proposes a new strategy for inhibiting this enzyme and should aid the further design of novel, potent and non-peptidic G6PDH inhibitors.

1. Introduction

Human glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) is a $NADP^+$-dependent homodimeric enzyme that catalyzes the transformation of D-glucose-6-phosphate into 6-phosphogluconolactone in the first step of the oxidative branch of the pentose phosphate pathway (PPP) [1]. This pathway generates NADPH molecules that protect the cell against oxidative stress. PPP also generates ribose-5-phosphate, an essential metabolite for nucleic acid synthesis. G6PDH has been reported as the enzyme with highest control coefficient over the flux of the oxidative branch of the PPP [2] and furthermore, several recent studies showed that G6PDH can be considered as a novel target for anti-cancer chemotherapy due to its essential role in maintaining the reduced antioxidant pool and in protecting the cell from oxidative stress [3–5]. Thus, throughout this branch ribose is synthesized and the reducing power necessary for fatty acid synthesis and for cell protection against oxidative damage is obtained [6,7]. Despite the importance of this enzyme no efficient inhibitors have been found up to date. Methotrexate [8] and dehydroepiandrosterone (DHEA) [9] are well known inhibitors of G6PDH, but the former is not specific and inhibits other $NADP^+$-dependent enzymes whereas DHEA, due to its hormone structure, affects many other cellular mechanisms and pathways. A novel class of compounds, represented by KPF-CoA, was recently discovered as G6PDH dimer disruptors [10], but they exhibited only modest activity. Additionally, combination of drugs such as cefaperazone/sulbactam [11] or ampicillin/sulbactam [11] inhibits G6PDH in a competitive and non-competitive manner, respectively, but little information is available about the binding site or mechanism of action of these compounds. Metamizol was also found to interfere with G6PDH activity [12].

As the crystal structure of human G6PDH is available [13], we carried out a molecular dynamics simulation to analyze the strongest contact points of the G6PDH homodimer, and further designed six interface peptides, including a cyclic peptide, as potential new inhibitors of the protein by disruption of the monomer–monomer interactions. The approach of targeting homodimeric enzymes has been recently reviewed [14].

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It is known that the $NADP^+$ cofactors stabilize the G6PDH homodimer structure and point mutations at the protein-protein interface affect the $NADP^+$ binding site and the enzyme catalytical activity [13,15]. It is therefore possible to inhibit G6PDH by distorting its dimer interface.

Peptides are ideal starting candidates to design inhibitors as they are able to interact within the extended, non-consecutive and flat surface of protein–protein interfaces [16]. Some examples of interface peptide inhibitors can be found elsewhere [17,18]. Recently, novel peptides with cytotoxic activity were designed by using information coming from molecular modeling [19]. Moreover, targeting protein–protein interactions to modify the interactome of the cell is changing the paradigm in drug discovery [20].

Although not presenting proper drug-like properties, peptides can be useful to further design small peptidomimetic molecules by retaining part of the interactions found in the peptide–protein system.

After predicting the affinity of six designed peptides by binding free energy calculations, three promising candidates were synthesized and one peptide of 14 residues was confirmed to be active in experimental assays. This peptide provides novel insights into the inhibition of human G6PDH.

2. Methods

All calculations described in the present study were carried out at the molecular mechanics level, using the amber force field [21] as implemented in the Amber 10 suite of programs [22]. The solvent was considered explicitly and the cut-off distance was kept to 9 Å to compute the non-bonded non-polar interactions. All simulations were carried out under periodic boundary conditions. Long-range electrostatic interactions were treated by means of the particle-mesh Ewald method [23] as implemented in Amber. All structural figures of the present study were prepared with the VMD software [24].

2.1. Preparation of the human G6PDH homodimer

The initial 3D structure of human G6PDH was downloaded from the Protein Data Bank, with entry code 1QKI [13]. As the minimum functional complex, we selected chains A and B for obtaining the G6PDH homodimer, due to the tetrameric structure of the enzyme is pH dependent presenting a rapid tetramer-dimer equilibrium [13]. The crystallized structure represents the G6PDH with the Canton mutation; this mutation was changed backwards in order to obtain the wild-type protein by using the Leap program of Amber. Glycerol and glycolic acid molecules were removed from the structure, whereas structural waters were maintained. Residues 1 to 26 of chain A and 1 to 27 of chain B were removed for presenting crystallographic data with low resolution. These residues are at an approximate distance of 30 Å from the dimer interface and thus we can assume that they have no influence in our simulations.

Leap program of the Amber suite was used to add hydrogen atoms, to neutralize the system with an appropriate number of sodium counterions, to solvate the system with a complete cubic box of TIP3P water model and to update the force field parameters of the $NADP^+$ cofactors, which were adapted from the Ryde set [25]. The final system consisted in G6PDH subunits A and B, two $NADP^+$ as cofactor molecules, eleven sodium counterions and a cubic box of 43,000 water molecules. Dimensions of the water box were $x = 107$ Å, $y = 126$ Å and $z = 105$ Å.

2.2. Molecular dynamics simulation

The G6PDH homodimer was energy-minimized in order to remove steric stress by means of a multi-step procedure. First, only water and counterion molecules were minimized constraining the remaining part of the system. Second, side chains of the protein were also minimized while protein backbone and cofactors remained constrained. Third, $NADP^+$ molecules were minimized as well as the protein backbone and finally all atoms were minimized by using 100,000 iterations combining the steepest descent and the conjugated gradient algorithms until a final energy gradient of 1.1 kcal/mol.

The minimized structure was subjected to a molecular dynamics simulation. It started by heating up the system to 300 K, coupling the system to a thermal bath with the Berendsen's algorithm [26] and a time coupling constant of 0.2 ps and incrementing the temperature using a constant rate of 30 K/10 ps. Once the density of the system was equilibrated by applying a pressure-constant step of 40 ps, molecular dynamics was carried out using the SHAKE algorithm [27] within the NVT ensemble along 2.5 ns of production run. Last nanosecond of converged total energy was used as production time for analyzing the stability of the protein–protein contacts.

2.3. Preparation and molecular dynamics of the peptide-G6PDH systems

Based on the equilibrated structure of the G6PDH homodimer, the starting structures of six peptide-G6PDH systems were prepared by retaining one subunit of the protein and utilizing the corresponding part of the second one for modeling each peptide. The $NADP^+$ molecule belonging to the entire monomer was retained. The cyclic peptide was derived from the natural sequence of the protein by adding two new glycine residues and creating the amide bond by means of the Leap program of Amber. After preparing all systems with the Leap program as described before for the G6PDH homodimer, they were submitted to equivalent minimizations (employing 50,000 iterations until energy gradients below 2 kcal/mol) and molecular dynamics simulations (2.5 ns of production run). Last nanosecond of converged total energy was used as production time for analyzing the stability of the peptide–protein contacts.

2.4. Binding free energy calculation of the peptide-G6PDH systems

The MMPB(GB)SA protocol [28] was used to predict the binding free energy of each peptide to G6PDH by extracting one hundred equidistant snapshots of each molecular dynamics simulation.

This method was used within the one-trajectory protocol that avoids calculating three separate molecular dynamics runs (for complex, ligand and receptor) and improves internal consistency. Molecular mechanics (MM) energies, representing internal, electrostatic and van der Waals components were extracted from the amber force field. Electrostatic solvation contribution to binding was calculated by means of the Poisson-Boltzmann (PB) or Generalized Born (GB) equations. For the latter, the parametrization of Tsui and Case [29] was utilized. Parse radii set was used both in PB and GB contributions. External and internal dielectric constants of 80.0 and 1.0, respectively, were used for the calculation. Non polar solvation effect was computed by using a linear relationship with the solvent accessible surface area (SASA) with a slope of 0.00542 kcal/mol$Å^2$ and 0.0072 kcal/mol$Å^2$ for the PB and GB contributions, respectively, and an intercept of 0.92 kcal/mol and 0.00 kcal/mol for the PB and GB contributions, respectively. Finally, entropic effects were computed through a normal mode analysis with the nmode module of amber before a minimization routine up to an energy gradient lower than 10^{-4} kcal/mol. Due to the computational cost of the entropy calculation, ten structures were extracted from the production dynamics and the G6PDH monomer was cut to only those residues located within a cutoff