Molecular docking studies on the DNA binding interactions, thioredoxinreductase and glutathione reductase of Alkynyl(triphenylphosphine) gold(I) complexes

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Abstract

Gold complexes as a new class of non-platinum antitumor drugs with prominent cytotoxic activities are presently being evaluated as potential anticancer agents. It was observed that the gold anticancer mechanism of action are through different pathways such as inhibition of the thioredoxinreductase (TrxR), selenium-glutathione peroxidase and glutathionereductase (GR) enzymes as well as intercalation with the DNA base pairs. Here, Molecular docking studies of Alkynyl(triphenylphosphine)gold(I) complexes, as novel anticancer gold(I) compounds were performed on three targets including TrxR, GR and DNA by means of AutoDock 4.2 to acquire the detailed molecular binding modes and binding sites for these compounds to the above targets. The docking results indicated that the important amino acids inside the active site of the cavity that are responsible for essential interactions are Ile A52, Glu A54 and Arg B25 for TrxR and Arg37, and Asn117 for GR receptors. A5, G4, G10 and C11 are among the most base pairs that involved in the interaction of these compounds to the DNA.

Keywords: Gold complexes, cytotoxic activities, Molecular docking.

Introduction

Platinum-based drugs have become a mainstay of cancer therapy(1-3). However, the platinum based treatment of tumoral diseases is immensely impeded by severe side effects and resistance development(4, 5). Among the non-platinum antitumor drugs, gold complexes are emerging as a new class of metal complexes with outstanding cytotoxic properties and are presently being evaluated as potential antitumor agents(6-8).

The antiproliferative mechanism of action of gold complexes had been under investigation for a long time. It was observed that many of the gold complexes inhibit the thioredoxinreductase (TrxR) enzyme with high specificity and potency and this target is the biological main target of gold complexes(9, 10).

The thioredoxin/thioredoxinreductase system (Trx/TrxR) which comprised of Trx, TrxR and NADPH, is an attractive drug target because of its involvement in various important physiological processes, containing DNA synthesis, antioxidant properties, redox homeostasis and regulating cellular viability and function. Hence, due to great relevance of TrxR for the
proliferation of tumor tissues, its inhibition is great importance in developing anticancer agents(10-12).

It should be mentioned that some gold complexes such as the gold phosphole complex GoPI or several gold(III) complexes interact with the DNA as their targets(13, 14).

The inhibition of selenium-glutathione peroxidase and glutationereductase (GR) was also reported for some gold complexes such as asuranofin, and aurothioglucose(7, 15). However these targets are more important for gold agents in the treatment of rheumatoid arthritis.

Recently, a class of Alkynyl(triphenylphosphine)gold(I) complexes has been synthesized and fully characterized by De Nisi, Assunta, et al. (16). Anticancer activity of these compounds were also investigated and it was concluded that their antiproliferative activity of them could be related to distinct mechanisms of action through different biological targets.

In the present paper, Molecular docking studies of Alkynyl(triphenylphosphine)gold(I) complexes, were performed on three targets including TrxRs, GR and DNA by means of AutoDock 4.2 to find out the detailed molecular binding modes and binding sites for these compounds interacting with the key residues of their targets.

Materials and Methods

**Data set:** A data set consisting of five Alkynyl(triphenylphosphine)gold(I) complexes as a series of potent antiproliferative agents were selected for the molecular docking study. The structural features of these compounds are shown in Scheme 1.

![Scheme 1. Chemical structure of Alkynyl(triphenylphosphine) gold(I) complexes used in molecular docking study](image)

**Docking procedure:** Two dimensional structures of all complexes were constructed using ChemBioDraw12.0 software(17). Each complexes was optimized by molecular mechanic methods (MM) using HyperChem 8(Version 8, Hypercube Inc., Gainesville, FL, USA), and then energy minimization calculations at Hartree-Fock (HF) level, using Gaussian 09. The output structures were thereafter converted to PDBQT using MGLtools 1.5.6.

The three dimensional crystal structure of DNA (PDB ID: 1BNA), TrxR(PDB ID: 4CBQ) and glutationereductase (PDB ID: 1BWC) were retrieved from protein data bank (http://www.rcsb.org/pdb/home/home.do). All water molecules were removed, missing hydrogens were added and after determining the Kollman united atom charges non-polar hydrogens were merged into their corresponding carbons using AutoDock Tools(18). As the final part of this process, desolvation parameters were assigned to each protein atom. Among the three different search algorithms performed by AutoDock 4.2 the commonly used Lamarckian Genetic Algorithm (LGA) was applied(19, 20). Subsequently, the enzymes and DNA were converted to PDBQT using MGLTOOLS 1.5.6.

The docking studies were carried out by means of an in house batch script (DOCKFACE)(21, 22) of AutoDock 4.2. For Lamarckian GA, a maximum number of 2,500,000 energy evaluations, 27000 maximum generations; 150 population size, a gene mutation rate of 0.02; and a crossover rate of 0.8 were applied. The grid maps of the receptors were calculated using AutoGrid tools of AutoDock 4.2. The size of grid was set in a way to include not only the active site but also considerable portions of the encircling surface. A grid box of 54×56×100,
50×50×50 and 50×50×50 points in x, y, and z directions was built and centered on the center of the ligand in the complex with a spacing of 0.375 Å for 1BNA, 4CBQ and 1BWC, respectively. Number of points for 1BNA in x, y and z was 14.719, 20.979 and 8.824, and for 4CBQ was 1.212, 0.263 and -0.383, and for 1BWC was 23.221, 63.866 and 20.642, consequently. AutoDock Tools was employed to produce both grid and docking parameter files i.e. gpf and dpf. Parameters for docking with metal ions such as gold, used in the docking calculation were added to gpf and dpf files. Parameters for docking with metal ions such as gold, used in the docking calculation were added to gpf and dpf files.

Cluster analysis was performed on the docked results using a root mean square deviation (RMSD) tolerance of 2 Å. For the internal validation phase, co-crystal ligand (auaranofin) inside the pdb file of TrxR (4CBQ) was extracted using a viewer and treated the same as other ligands. All the docking protocols were done on validated structures with RMSD values below 2 Å.

Ligand-receptor interactions were all detected on the basis of docking results using Autodock tools program (ADT, Version 1.5.6), VMD software(23), and PyMOL molecular graphics program(24).

All calculations were run on a core i7 personal computer (CPU at 8 MB) with Windows 7 operating system. With respect to the AutoDock scoring function, the lowest docking binding energy conformation was chosen as the best binding mode.

Results and Discussion

Here, molecular docking studies were performed on the Au(I) complexes to find their binding site, binding modes and the best direction on the base of their binding energy to DNA, TrxR and GR. The results obtained from this part of study including the estimated free binding energy values (ΔG bind) for the best position of the docked compounds, expressed in kcalmol⁻¹(Top ranked binding energies (kcal/mol) in AutoDockdlg output file were considered as response in each run), along with the corresponding favorable interactions with the key amino acid residues at the active site of enzymes and the base pairs of DNA are summarized in Table 1 and figures 1-3.

Table 1. The docking binding energies of compounds 1a-e on DNA, TrxR and GR as well as their biological activities.

| Name | ΔEa (kcal/mol) | DNA | TrxR | GRb | Exp. IC50 (µM)c | %TrxR inhibitiond,e |
|------|---------------|-----|------|-----|----------------|---------------------|
| 1a   | -7.14         | -7.49| -7.34| 20  | 33             |
| 1b   | -9.97         | -6.86| -9.16| 5.3 | 3.3            |
| 1c   | -9.42         | -7.12| -8.97| 5.5 | 2.7            |
| 1d   | -8.69         | -8.31| -8.13| 6.5 | 6.3            |
| 1e   | -8.21         | -8.62| -7.86| 10  | 9              |

a docking binding energy
b Data was extracted from De Nisi, et al. article (16).
c The inhibitory effect on TrxR is expressed as % of inhibition taking as reference the auranofin maximal inhibition (100%)
d Glutathionereductase
e human ovarian adenocarcinoma line
f human Caucasian promyelocytic leukemia
g Not determined

The ΔG bind values of the best docked poses of compounds 1a-1e are within the range of -7.14 to -9.97 kcal.mol⁻¹ for DNA, -6.86 to -8.62 kcal.mol⁻¹ for TrxR and -7.34 to -9.16 kcal.mol⁻¹ for glutathione reductase.

As it was shown in table 1, complex 1b has the greatest binding energy on DNA and glutathione reductase, whereas it has the lowest binding energy on TrxR. These data is in good accordance with biological activity which showed that 1b couldn’t be considered as an inhibitor of this class of enzymes.

The results of molecular docking simulations on DNA and GR targets were highly in accordance with experimental cytotoxic data on cancerous cell lines (Table 1).

Docking validation step was done by re-docking of the co-crystallized conformation of ligands into 3D structure of TrxR (auranofin is the co-crystal ligand inside the pdb file of TrxR (4CBQ)). Generally, if the root mean square deviation (RMSD) is below 2 Å, it is considered a successful prediction. All the docking protocols were done on validated structures with RMSD values below 2 Å. With respect to the negativity values of the binding free energy, it can be deduced that these complexes reasonably bind to the these targets.
The docked model suggests that the most desirable conformation of the docked pose of 1b from energetically point of view interacts with the minor groove of 1BNA (figure 1). It interacts via one of the phenyl group of its triphenylphosphine (PPh₃) moiety with G10 through arene-H binding interaction. thiol group with G4 and phenylpyridine moiety with C3 base pairs in the minor groove of DNA. The oxygen atoms of its sulfonamide moiety are involved in acceptor-type hydrogen bond with G4 and A5 base pairs. A weak interaction between methyl attached to the nitrogen with C11 was also observed (figure 1).

**Figure 1.** Molecular docking simulation studies of the interaction between 1b and DNA (PDB ID: 1BNA)

Binding mode of compound 1b with TrxR (figure 2) shows that oxygen group of its sulfonamide are involved in acceptor hydrogen bond with Glu A54 amino acid. An arene-hydrogen interaction between the phenyl of phenylsulfonamide moiety and residue Ile A52 was observed. It is also interact via its sulfonamide nitrogen with residue Arg B25. The methyl attached to the nitrogen is in the interaction with Glu A54.

As indicated in figure 3, the oxygen atoms of sulfonamide are involved in hydrogen bond interactions with Arg37. The phenylring attached to the sulfonamide group, is involved in interactions with Asn117 amino acid.
Conclusion

Gold complexes are a new class of non-platinum anticancer agents with outstanding cytotoxic activities. Inhibition of the thioredoxin reductase (TrxR), selenium-glutathione peroxidase and glutathione reductase (GR) enzymes as well as intercalation with the DNA base pairs are among the most accepted mechanism of action of gold anticancer agents.

In this paper, to obtain the detailed molecular binding modes and binding sites for gold anticancer agents, novel anticancer gold(I) compounds, Alkynyl(triphenylphosphine)gold(I) complexes, were subjected to molecular docking studies on three targets including TrxR, GR and DNA by means of AutoDock 4.2. The docking results indicated that the important amino acids inside the active site of the cavity that are responsible for essential interactions are Ile A52, Glu A54 and Arg B25 for TrxR and Arg37, and asn117 for GR receptors. A5, G4, G10 and C11 are among the most base pairs that involved in the interaction of these compounds to the DNA.

As an overall result these studies suggest that in addition to inhibition of TrxR and GR, the interaction with various biomolecules such as DNA seems to be important for the pharmacology of anticancer gold complexes.
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