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Molecular docking study investigating the possible mode of binding of C.I. Acid Red 73 with DNA

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ABSTRACT

C.I. Acid Red 73 is a reactive azo dye with a variable potential carcinogenicity. The mechanism mediating interactions that occur between the dye and DNA have not been completely understood thus far. In this study, molecular docking techniques were applied to describe the most probable mode of DNA binding as well as the sequence selectivity of the C.I. Acid Red 73 dye. These docking experiments revealed that the dye is capable of interacting with the minor groove of the DNA on the basis of its curved shape, which fits well with the topology of double-stranded DNA. In addition, the dye can bind selectively to the minor groove of the DNA by applying CGT sequence selectivity. Further, the minor groove can be recognized although DNA targets present intercalation gaps. However, intercalative binding can also occur when the DNA target possesses an appropriate intercalation gap. Compared with the other eight DNA sequences that were studied, the DNA dodecamer $d(CGCGATATCGCG)_2$ (PDB ID: 1DNE) presents a very favorable target for the binding of C.I. Acid Red 73 to the minor groove, with the lowest binding free energy −9.19 kcal/mol. Results reported from this study are expected to provide useful information for research involving further simulations of molecular dynamics and toxicology investigations of the dye.

1. Introduction

In the textile industry, the dyeing process generates a large volume of wastewater which contains most of the dyes that have remained non-reactive during processing stages [1]. The discharge of this wastewater into water bodies not only damages aquatic plants and animals but also causes serious environmental problems [2–4]. Different classes of dyes are used in industrial applications, and azo dyes are used most frequently, thus constituting 60–70% of all dyes employed [5–7]. Several synthetic azo dyes and their metabolites have been found to manifest toxic, carcinogenic, and genotoxic effects [8–12].

C.I. Acid Red 73 is a reactive azo dye that has two sulfonic groups, and its molecular structure is presented in Fig. 1. Sulfonated azo dyes are usually difficult to remove during wastewater treatment and, therefore, they might contaminate surface waters because of their high water solubility [13]. There is evidence that sulfur-containing dyes possess genotoxic effects [14–18]. Therefore, dyes that bind with the DNA have become the focus of exigent research with regard to its association with genetic effects and carcinogenicity [19].

Although C.I. Acid Red 73 has been banned for use in hair-coloring products by the European Commission (http://www.care2.com/green), it is used in textile-dyeing processes because of its dyeing efficiency and low costs incurred by its use. In a previous study, these authors have reported the molecular mechanism involved in the binding of the dye with human serum albumin [20]. In addition, the biotoxicity of dyes was evaluated with bioluminescence tests. Results demonstrated that C.I. Acid Red 73 can inhibit bacterial luminous activity and alter the conformation of biomacromolecules. However, the detailed mechanism involved in the binding of the dye with DNA has not been reported previously. A clear understanding of the molecular mechanism involved when C.I. Acid Red 73 binds with DNA might facilitate definition of the carcinogenic toxicity potential of the dye.

Small molecules that bind with macromolecules play a key role in biological processes; this applies more specifically to molecules that bind with various morphologies of nucleic acids at multiple sites to modify nucleic acid function [21–24]. In certain cases, interactions between potential ligands and DNA result in structural distortion or damage of DNA. In other cases, the binding may interrupt replication and transcription of a specific gene or, ultimately, induce cell death [25].

The dominant binding modes of small molecules with DNA can be categorized into two major classes: (i) covalent binding, and (ii) noncovalent binding, which includes intercalative binding and DNA

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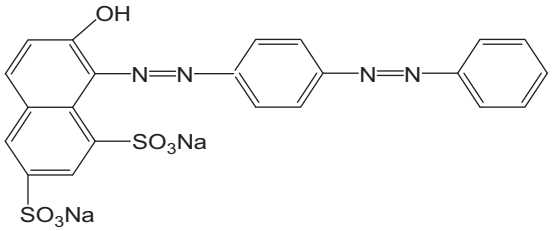

Fig. 1. Molecular structure of C.I. Acid Red 73.

major/minor groove-binding [26]. Intercalation and minor-groove binding constitute the predominant DNA-binding modes of small ligands [27–30].

In research exploring the importance of dye–DNA interaction in carcinogenesis, investigations employing molecular docking techniques tend to focus on unraveling the nature of the binding by C.I. Acid Red 73 with DNA. Molecular docking studies provide some insight into interactions between potential ligands and their macromolecular targets [31]. Computational methods are playing an increasingly larger and more important role in drug discovery and development [32]. Further, they are expected to provide helpful information with regard to molecular toxicology and, thereby, significantly decrease traditional resource requirements encountered in biological testing.

In this context, the software AutoDock 4.0 was selected for use in the present study to perform flexible ligand docking between C.I. Acid Red 73 and nine structurally distinct DNA sequences. The most probable DNA-binding modes and sequence-selective binding of the dye were identified by comparing predicted binding affinity and the preferred orientation. These predicted binding modes may provide a structural basis for understanding the interaction of C.I. Acid Red 73 with DNA and offer potential for the interpretation of the toxicity of the dye at the molecular level.

2. Methods

Molecular docking was accomplished by the molecular docking software, AutoDock Version 4.0 – a software program applying an empirical scoring function that is based on the binding free energy of the complex. This method allows automated docking of fully flexible ligands to the rigid macromolecular receptor. Four files are required to be prepared before initiation of docking.

2.1. Preparation of the macromolecule

Crystal structures of nine DNA ligand complexes from the Protein Data Bank (PDB IDs: 1DNE, 102D, 1MKL, 1DC0, 1D32, 1ZNA, 1Z3F, 1K2J, and 1MTG) were selected. The ligand and all the water molecules were removed by the software program UCSF Chimera. Polar hydrogens and Gasteiger charges were added after merging non-polar hydrogens. Bonds were built based on distance, and atoms were assigned to the AD4 type. The charge field was set to the Gasteiger charge, and the partial atomic charges were calculated using the Gasteiger–Marsili method, during the preparation of the macromolecule input file, by using the AutoDockTools package.

2.2. Preparation of small molecules

The three-dimensional (3D) structure of C.I. Acid Red 73 was determined using GaussView 3.07 (www.gaussian.com). The geometry of the ligand was optimized using the software program Gaussian 03 [33] at the level of B3LYP/6-31G(d). Gasteiger charges were added and the rotatable bonds were set to 7 by using AutoDock Tools.

2.3. Preparation of the grid file

Grid maps were generated with the AutoGrid (part of the AutoDock package). The points of the grids were $60 \times 60 \times 120$ for 1DNE and 102D, $60 \times 70 \times 110$ for 1MKL, $90 \times 90 \times 120$ for 1DC0, $56 \times 44 \times 60$ for 1D32 and 1ZNA and $60 \times 60 \times 80$ for 1Z3F and 1MTG, respectively, with a grid spacing of 0.375 Å.

2.4. Preparation of the docking file

Automated docking studies were carried out using AutoDock version 4.0. Among the three different search algorithms offered by AutoDock 4.0, the Lamarckian genetic algorithm (LGA) was used to identify appropriate binding modes and conformations of the ligand. During each docking experiment, 50 independent docking runs were performed for each docking case containing a population of 150 randomly positioned individuals. The maximum number of energy-evaluation retries and generations were 2 500 000 and 27 000, respectively, and miscellaneous parameters were selected as default values. Cluster analysis was performed on the results of docking by using a root mean square (RMS) tolerance of 2.0 Å, and this was dependent on the binding free energy.

2.5. Calculation of the binding free energy

The binding free energy was calculated using the SSH Shell software on the Red Hat Enterprise VMware player workstation using the equation [34]

$$\text{Binding free energy} = (1) + (2) + (3) - (4)$$

where (1) is the final intermolecular energy (vdW + H bond + desolv energy + electrostatic energy); (2) is the final total internal energy; (3) denotes the torsional free energy; and (4) represents the energy of the unbound system. The mean binding free energy was calculated based on the binding free energy of the five optimum binding modes.

3. Results and discussion

3.1. Identifying of minor/major groove-binding mode

Molecular docking experiments were performed, using the docking protocol described in Section 2, in order to identify the binding site and the preferred orientation of C.I. Acid Red 73 within the DNA minor/major groove. Based on the structural features of C.I. Acid Red 73, two models were considered to be possible DNA-binding modes: (i) minor/major groove binding and (ii) intercalation binding. In order to identify the DNA minor/major groove-binding mode, two crystal structures of the DNA dodecamer d(CGCGATATCGCG)$_2$ (PDB ID: 1DNE) and d(CGCAAATTTGCG)$_2$ (PDB ID: 102D) were selected as the groove-binding model for the docking studies. Both of these dodecamers possess two long CG and a long AT tract of base pairs in length with several potential binding sites in the DNA groove.

During each docking experiment, one ligand conformation with the lowest docking energy was selected from the 50 ligand conformations for further analysis of results and plotting of graphs. Docking results demonstrated that 50 ligand conformations were completely docked to each minor groove of the two distinct DNA segments. The optimum binding modes of C.I. Acid Red 73 with DNA (1DNE and 102D) are displayed in Fig. 2(a) and (b). The base pairs adjacent to the dye on the binding sites are depicted in Fig. 3(a) and (b). Data obtained on the optimum binding conformations are shown in Table 1.