Journal of Fundamental and Applied Sciences

Research Article

ISSN 1112-9867

Available online at http://www.jfas.info

DETERMINATION OF THE BINDING PARAMETERS OF THE ADDUCT 3-NITRO PHENYLFERROCENE-DNA USING CYCLIC VOLTAMETRY, ELECTRONIC SPECTROSCOPY AND MOLECULAR DOCKING SIMULATION

H. Ben amara*, T. Lanez

University of El Oued, VTRS Laboratory, B.P.789, 39000, El Oued, Algeria

Received: 28 May 2019 / Accepted: 25 August 2019 / Published online: 01 September 2019

ABSTRACT

Binding parameters of the interaction of 3-Nitrophenylferrocene ($3FcNO_2$) with DNA, has been measured by cyclic voltametry (CV) and electronic spectroscopy (Uv-Vis). The obtained results were validated by molecular docking (MD) using AutoDock Tools software. CV results further indicated that 3FcNO2 interact to DNA by hydrogen bonding with binding constant and binding free energy values equal to 7.59×10^{4} M^{-1} and -27.85 $KJ.mol^{-1}$ respectively. These values were in a good agreement with those obtained from Uv-Vis measurements. The diffusion coefficient values of the free and bounded $3FcNO_2$ with DNA obtained from CV experiments were respectively equal to 9.71×10^{-8} and 4.33×10^{-9}, this finding further confirm the formation of $3FcNO_2$-DNA adduct. Binding parameters were also estimated by MD, the obtained values were identical to experimental results.

Keywords: $3FcNO_2$, cyclic voltammetric, DNA, UV-spectroscopic, molecular docking.

Author Correspondence, e-mail: benamarahacen@gmail.com

doi: http://dx.doi.org/10.4314/jfas.v11i3.15

1. INTRODUCTION

In organometallic compounds group, ferrocene has significant interest, mainly due to transitions p-electrons of ferrocene that conform to an empirical the eighteen-electron rule for

transition metal metallocenes [1], stability of chemical structure and its toxicity is low. It has been exploited to make drug design requirements for the pharmacophore industry [2] .Much scientific and applied studies have been executed in recent years to determine its structural properties [3]. Common applications of Ferrocene were also suggested in the medical sciences which describe materials as well as for studying the movement of electrons [4]. Furthermore, derivatives of ferrocene compound are also known for their antibacterial [5], antioxidant [3] and anticancer activities [2]. Our concern in 3-Nitrophenylferrocene, So far there is no study has been achieved on their interaction with DNA and we can use this type of compound to treat mutations that can occur on double helix structure of DNA [5-6].

generally, the various positions on sequences of nucleic acids can interacting with studied compound by specific types of bonds can be between the DNA and ferrocene compound that is a ligand-mediated interactions due hydrogen bonds; and second type direct ferrocene derivate bonding with DNA by a van der Waals interactions, it are attributed to different polarity of nucleobases' atoms [7].

Moreover, $3FcNO_2$-DNA interactions have been investigated via a variety of analytical techniques, are cyclic voltammetric method and absorption UV–visible spectroscopy [8-9]. these techniques are most used to find out a nature of interaction drug–DNA [8], as well as for quantitative study for samples with a function of various concentrations of DNA [10], within limits a small concentrations from 1.63µM to 14.29µM, Cyclic voltammetry (CV) was used to control the substantial-decrease of the anodic current peak by Successive additions of DNA [3-6]. To explore characterizations of interaction with DNA for synthesized compound ($3FcNO_2$) can be used CV and electronic spectroscopy Assays [6].

2. MATERIAL AND METHODS

2.1 Chemicals and reagents

3-Nitrophenylferrocene was synthesized according to the literature procedure [11]. Electrochemical and spectroscopic data were similar to the proposed structure of $3FcNO_2$.

2.2. DNA extraction

DNA were extracted from chicken blood samples using flacon method, the obtained DNA was quantified by UV absorbance at $\lambda = 260$ nm. and stored at 4 °C [12-15].

2.3. Instrumentation and software

CV studies were performed on a PGZ301 potentiostat/galvanostat (radiometer analytical SAS (France)) in a conventional three electrode cell composed a glassy carbon (GC) with a geometric area of 0.013 cm^2 as working electrode and an Hg/Hg_2Cl_2 reference electrode containing 3.0 M KCl and the counter electrode was a platinum wire. The redox behavior of 1 mM of $3FcNO_2$ in the absence and presence of DNA were presented in Figure1a.

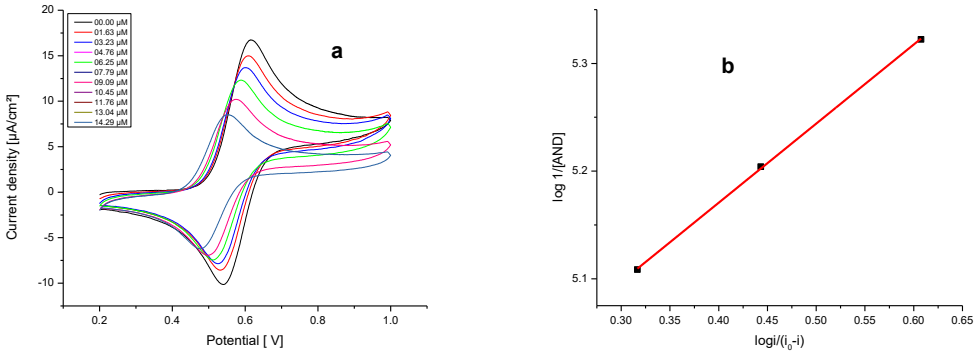

Fig.1. CV behaviors of 1 mM of $3FcNO_2$ in DMF in the absence and presentence of DNA, at 0.1 V/s scan rate

Figure 1b shows the plot of $1/1-(i/i_0)$ versus $1/[DNA]$ using the following equation (1) [16]

$$\log \frac{1}{[DNA]} = log\ K + log\ \frac{i}{i_0 - i} \qquad (1)$$

The plot of $1/1-(i/i_0)$ versus $1/[DNA]$ was exploited to determine the binding constant of the adduct $3FcNO_2$- DNA.

3. RESULTS AND DISCUSSION

3.1. CV study

3.1.1. Binding constant and free energy

These were determinate from the shift in peak current densities of the voltammograms presented in figure 1a. [17].

Table 1. Value of binding constant and binding free energy obtained from CV Measurements

Adduct	Equation	R^2	$K(M^{-1})$	$-\Delta G$ (KJ mol^{-1})
$3FcNO_2$-DNA	y = 0.73x + 4.88	0.99	7.59×10^4	27.85

ΔG is calculated using equation 2 [18]:

$$\Delta G = -R\,T\,ln \qquad (2)$$

Where, ΔG indicates the Gibbs free energy for binding in KJ mol^{-1}, R is the universal gas constant (8.32 J mol^{-1} K^{-1}) and T is the absolute temperature (298 K).

The equation presented in Table 1 was obtained by the straight line graph in the range of studied concentration. Data in Table 1 further indicate that the binding was electrostatic and spontaneous [19,20].

3.1.2 Diffusion coefficients

CV voltammograms of $3FcNO_2$ in the absence and presence of DNA at different scan rates are presented in figure 2.

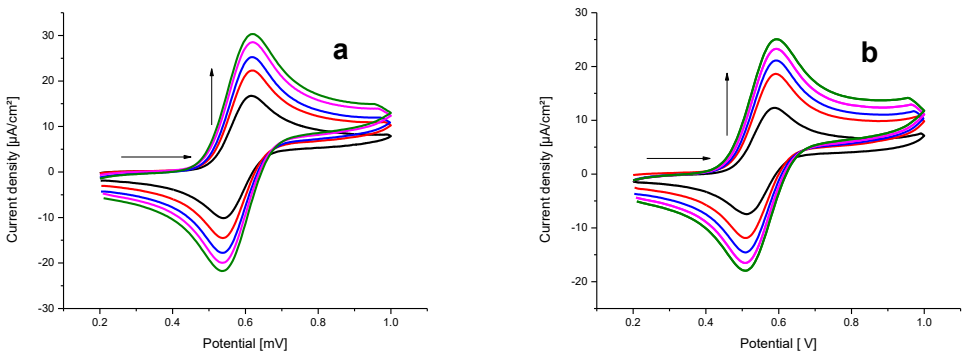

Fig.2. CV behavior of 1 mM $3FcNO_2$ on glassy carbon electrode in the absence (a) and presence (b) of 6.25 µM DNA ; scans rates 500, 400, 300, 200 and 100 mV. s^{-1} with supporting electrolyte 0.1 M TBATFB. vertical arrowhead indicates increasing scan rate

Anodic peak current versus the square root of scan rate plots for $3FcNO_2$ (Figure 3), indicate that the redox process is diffusion limited. Values of coefficient difusion of the free and DNA bound $3FcNO_2$ were deducted using the slopes of the linear regression of the Randles-Sevcik equation 3 [21-23].

$$i = 2.69\times10^{5} n^{\frac{3}{2}} SCD^{\frac{1}{2}} v^{\frac{1}{2}} \qquad (3)$$

where n is the number of electrons per species reaction , S is the apparent surface area of the electrode (cm^2), D is the diffusion coefficient of $3FcNO_2$ ($cm^2.s^{-1}$) and C is the concentration of $3FcNO_2$ ($mol.cm^{-3}$), ν the scan rate ($V.s^{-1}$).

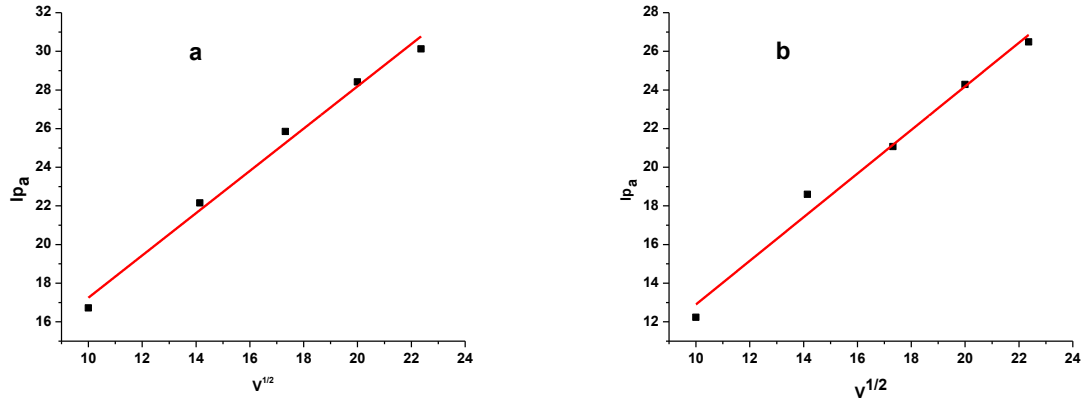

Fig.3. Plots of i vs. $v^{1/2}$ of 1 mM $3FcNO_2$ in the absence (a) and presence of 6.25 µM DNA (b)

Table 2 resumed the obtained diffusion coefficients for the free and bounded $3FcNO_2$.

Table 2. diffusion coefficient values of the free and bounded $3FcNO_2$

Adduct	Equation	R^2	$D(cm^2/s)$
$3FcNO_2$	y = 1.09x +6.30	0.98	9.71×10^{-8}
$3FcNO_2$-DNA	y = 1.12x +1.61	0.98	4.33×10^{-9}

3.2. Uv–Vis studies

The Uv-Vis absorption titration method was used to confirm the previous results obtained from CV study. 1mM solution of $3FcNO_2$ in DMF has been titrated by the incremental additions of DNA, in Figure 4.

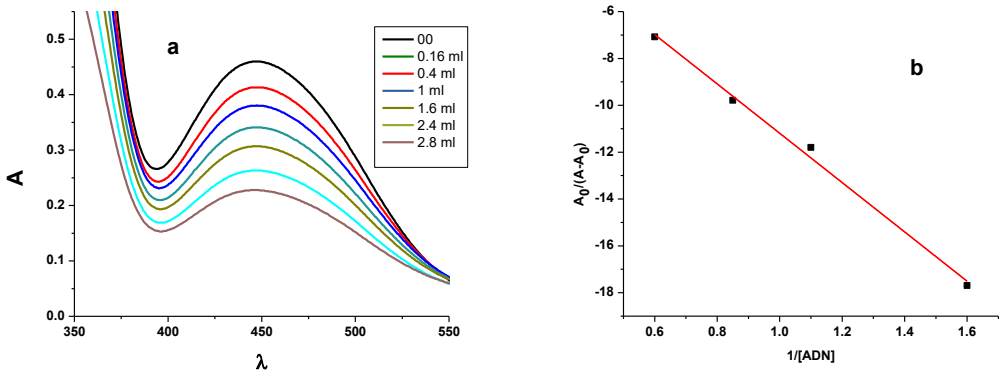

Fig.4. (a) Absorption spectra of 1mM solution of $3FcNO_2$ in DMF in the absence and presence of DNA, (b) plots of $A_0/(A - A_0)$ vs. 1/[DNA]

Based upon the change in absorbance values, the binding constant (K) of the interaction of the ligand $3FcNO_2$ with DNA was be calculated according to Benesi–Hildebrand equation 3 [24,25],

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_0}{\varepsilon-\varepsilon_0} + \frac{\varepsilon_0}{\varepsilon-\varepsilon_0}\frac{1}{K[DNA]} \qquad (3)$$

where A_0 and A are absorbance s of $3FcNO_2$ in absence and presence of DNA, respectively, and ε_0 and ε are their respective absorbance coefficients, [DNA] is DNA concentration, K is binding constant. K was obtained from the ratio of intercept to slope of the plot of $A_0/(A - A_0)$ versus 1/[DNA], results are presented in Table 3.

Table 3. Values of binding constant and binding free energy

Adduct	Equation	R^2	K(M-1)	-ΔG (KJ mol−1)
$3FcNO_2$-DNA	y = -10.52x – 0.66	0.99	6.27×104	27.39

3.3. In silico analysis

Docking simulation was used to get more information about the interaction of 3FcNO2 with DNA and to validate the previously obtained results from CV and Uv-Vis measurements.

3.3.1. Structure optimization

Structure optimization of $3FcNO_2$ was carried out using Gaussian 09 program [26] using the density functional theory (DFT) at B3lyp/6-31+G(d,p) basis set for all atoms [27], by

default at temperature room 298K. The optimized structure is shown in Figure 5.

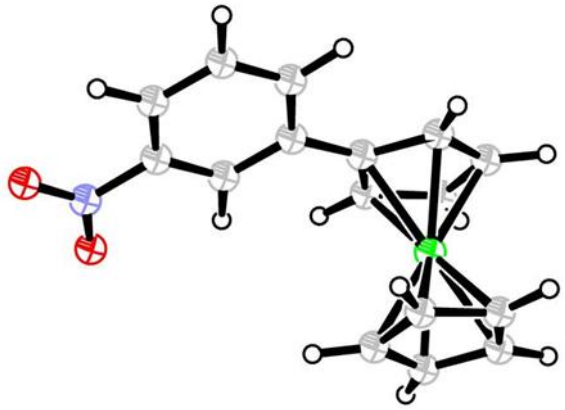

Fig.5. Optimized structure of $3FcNO_2$ (ORTEP View 03, V1.08); color codes are gray carbon, white hydrogen, blue nitrogen, and red is oxygen

3.3.2 Computational docking study

Docking study was carried out using AutoDock 4.2.6 software (version 1.4.5) [28] and performed on a windows 7 operating system with an Intel Pentium 3.30 GHz and RAM 4.00 Go microcomputer MB memory. The crystal structure of DNA (PDB ID: 1BNA) of sequence: $D(CGCGAATTCGCG)_2$ was taken from the Protein Data Bank **(**http://www.pdb.org**)** [29].

For docking section, Lamarckian genetic algorithms used a number of grid points of box enclosing the DNA, values were as follows 14.78 x 20.976 x 8.807 with point number separated by 1.000 Å and a grid spacing of 0.3751 Å, the size of the grid box was set at x = 50,y = 60 and z = 90. Single docking experiment consisted of 60 docking runs with 150 random individuals and maximum number of 2,500,000 energy evaluation; the other parameters were left without changing. 41 Points for grid per dimension it have been used to carried out the search and a step size of 0.375 centered on the binding site of $D(CGCGAATTCGCG)_2$, the best docked conformation was finally chosen based on the lowest binding energy [30,31].

After completing docking run, various ΔG values were obtained with their docked conformations; a computational ligand-target docking approach of stalest conformation for best pose was used to analyze structural complex of the DNA (target) with $3FcNO_2$. Figure 6 shows the ligand $3FcNO_2$ is placed in the DNA structure. The binding free energy was −27.71

KJ.mol^{-1} at 47th run. This value is very close to the experimental values obtained from CV and Uv-Vis measurements. A magnitude of the binding free energy illustrated a high binding affinity of the 3FcNO2 to DNA. In addition, the binding constant is determined from equation 2 cited previously.

Table 5. Values of free binding energy and binding constant calculated for 3FcNO$_2$-DNA adduct by molecular docking simulation

Complex	K(M^{-1})	-ΔG (KJ mol^{-1})
3FcNO$_2$-DNA	7.14x10^4	−27.71

DNA is shown in figure 8 as orange, yellow, red and blue thin-tube of DT, DG, DC and DA, respectively. The ligand is displayed as stick; with color codes of atoms are C, cyan, O, red; N, dark blue and P, orange. Hydrogen atoms have not been shown to achieve lucid visualization. The ligand and target are separated by some physical distance and interact by means of mostly H-bond.

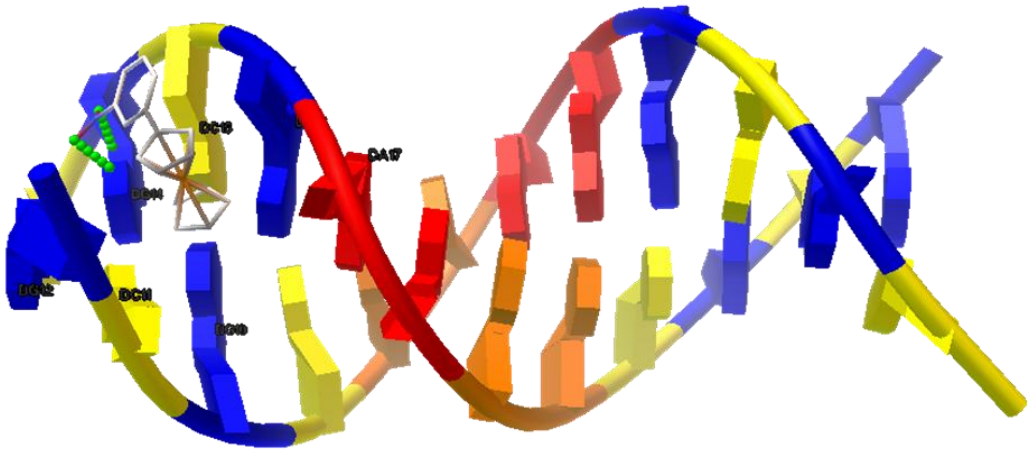

Fig.6. Docking pose of 3FcNO$_2$in the active site of DNA (PDB ID: 1BNA) showing the interaction between DNA and the ligand

Furthermore, docking results clearly shown the formation of two hydrogen bonds between the oxygen atom of 3FcNO$_2$ and the hydrogen atoms of (DG14:H22/DG14:H3) (Figure 7).

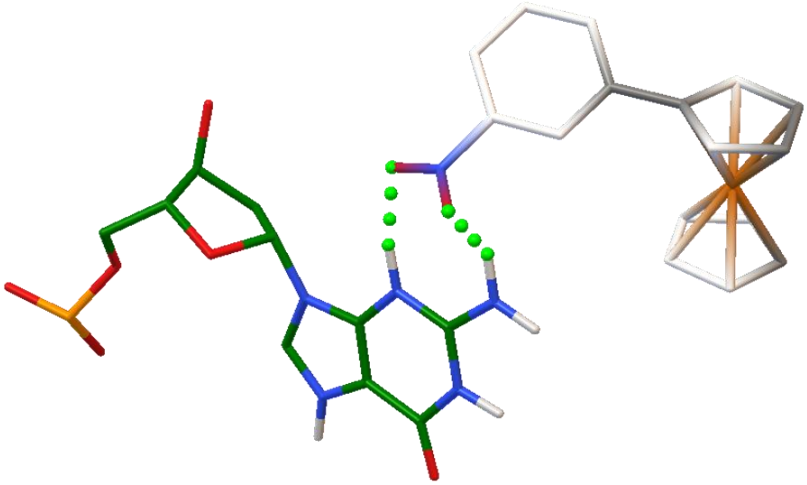

Fig.7. Hydrogen bonding between $3FcNO_2$ and DNA nucleotides (The small green spheres show H-bond interaction)

Figure 8 illustrates the surface view of the adduct $3FcNO_2$-DNA, it shows that the ligand is placed in the minor groove of DNA by non-covalent interactions is presented. For clarity, segment from DNA template, has been omitted.

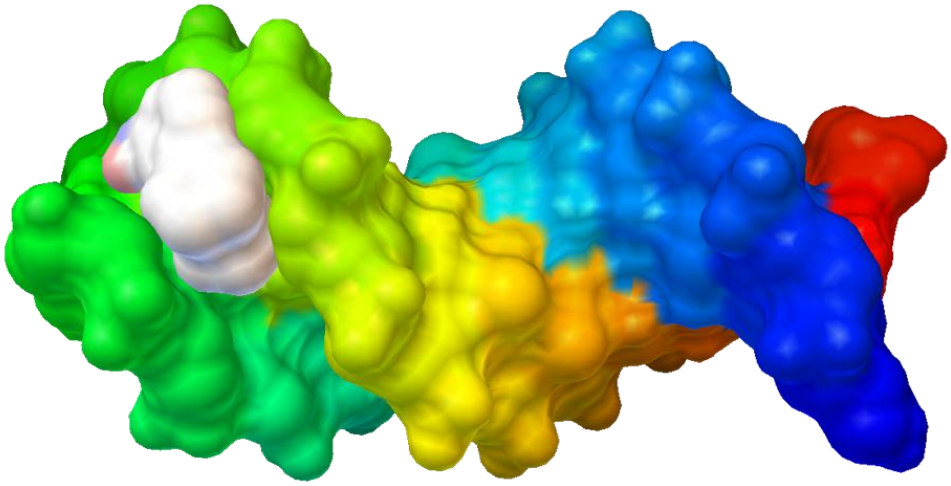

Fig.8. Surface view of docked $3FcNO_2$ with DNA (PDB ID: 1BNA)

4. CONCLUSION

The binding parameters such as binding constant and binding free energy of the interaction of the ligand $3FcNO_2$ with DNA was determinate using cyclic voltammetry and electronic

spectroscopy assays, both assays gave the same values which were in a good agreement with those obtained by molecular docking. Molecular docking further indicates that the ligand $3FcNO_2$ interacts with DNA by the formation of two hydrogen bonds formed between its oxygen atom and the hydrogen atoms of (DG14:H22/DG14:H3).

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How to cite this article:
Ben amara H, Lanez T. Determination of the binding parameters of the adduct 3-nitro phenylferrocene-dna using cyclic voltametry, electronic spectroscopy and molecular docking simulation. J. Fundam. Appl. Sci., 2019, *11(3), 1267-1278.*