INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 4, p. 27-33, 2014

RESEARCH PAPER OPEN ACCESS

Spectroscopic studies on the interaction between acridine-spermine conjugate with DNA

Masoud Karimi Goftar[1*], Narges Alizadeh Rayeni[1], Neda Mohamadi[2]

[1] *Young Researchers and Elite Club, Baft Branch, Islamic Azad University, Baft, Iran*

[2] *Young Researchers and Elite Club, Kerman Branch, Islamic Azad University, Kerman, Iran*

Key words: Acridine-spermine, spectroscopic study, MCR-ALS, equilibrium constant, thermodynamic parameters, vant-hoff equation.

http://dx.doi.org/10.12692/ijb/5.4.27-33 Article published on August 18, 2014

Abstract

Interaction of acridine-spermine with chicken blood ds-DNA has been investigated via spectrophotometric method at different temperatures. The equilibrium constants of acridine-DNA complex in several temperatures have been determined by applying MCR-ALS method on the absorption spectra. The quantitative analysis of the data of undefined mixtures, was carried out by simultaneous resolution of the overlapping spectral bands in the whole set of absorption spectra. The enthalpy and entropy of the dimerization reactions were determined from the dependence of the equilibrium constants on the temperature (van't Hoff equation). From these results it can be inferred that the driving force of the equilibrium is of enthalpic origin.

* **Corresponding Author:** Masoud Karimi Goftar ✉ karimi.goftar@yahoo.com

Introduction

Acridine is a heteroaromatic molecule able to intercalate into DNA (Demeunynck *et al.,* 2001). This structural property confers anti-tumour capabilities to acridine and its derivatives, since DNA is the main target in anti-cancer drug design (Graves *et al.,* 2000). Intercalation of a planar system between DNA base pairs can induce structural alterations in such a way that cell proliferation is blocked (McDonald *et al.,* 1994; Dias *et al.,* 2004). A usual mechanism to explain this fact is related to the DNA-binding enzymes which have essential roles in reactions directly involved in cell proliferation, such as topoisomerases and telomerases (Larsen *et al.,* 2003). The DNA-intercalator complexes prevent, in most cases, DNA recognition by these enzymes, thus disrupting the whole process.

Transcription and replication are not only important for cell survival and proliferation but they also help in smooth functioning of all body processes. DNA starts transcribing or replicating only when it receives a signal, which is often in the form of a regulatory protein binding to a particular region of the DNA. Thus, if the binding specificity and strength of this regulatory protein can be mimicked by a small molecule, then DNA function can be artificially modulated, inhibited or activated by binding this molecule instead of the protein. The synthetic or natural small molecule can act as a drug when activation or inhibition of DNA function is required to cure or control a Disease (Uil *et al.*, 2003). Chemotherapy is the technique in which replication of DNA is stopped by these foreign molecules i.e., drugs. Reactivity of deoxyribonucleic acid (DNA) offers the analytical chemist a powerful tool in the recognition and monitoring of many biologically important compounds (Palecek *et al.,* 1998). DNA contains all of the genetic information related to cellular function, including DNA replication and gene expression. However, DNA molecule can be easily damaged, e.g., by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Oliveira *et al.*, 2004). Therefore, in a health preventing perspective, there is a deep interest in identifying free radical scavengers or antioxidants that inhibit oxidative DNA damage.

It is known that one of the main problems of anti-tumour therapies is the secondary effects caused by the action of cytotoxic drugs on healthy cells. Most of the promising drugs preserve cytotoxic activity, although they have tumour cells as their main target. In the case of intercalators, this selectivity can be acquired by attaching an aliphatic side chain (Graves *et al.,* 2000). These chemical substituents can also provide both cell-specificities and new contributions to DNA binding, as they can establish additional interactions with intra-cellular reactive sites. As a consequence, changes can appear in the thermodynamic parameters of binding and in useful biochemical properties, such as uptake systems or sequence selectivity. This strategy is being currently tested by functionalizing the acridine molecule (Demeunynck *et al.,* 2001). Acridine based anti-tumour drugs have been developed and studied (Oliveira *et al.*, 2004), as amsacrine or asulacrine, which present excellent activity against some cases of leukaemia (Oliveira *et al.*, 2004). Acridine derivatives recently synthesized have been proven to stabilize G-quadruplex structures in telomeres, inhibiting telomerase action (Harrison *et al.,* 2003).

The biogenic polyamines are organic polycations essential for cell proliferation. Tumour cells uptake biogenic polyamines in greater amounts than do healthy cells (Graves *et al.,* 2000), thus making them suitable candidates for improving the anti-cancer action of intercalators like acridine. Three important features have been recently proven for a series of acridine–polyamine covalent conjugates (Harrison *et al.,* 2003): i) they present higher affinities for DNA than acridine; ii) they preserve the topoisomerase II inhibitory activity of acridine and, iii) they are recognized by the polyamine transport system. Acridine–polyamine conjugates are, therefore, promising chemical systems for the development of more efficient anti-tumour drugs. Nevertheless, this task requires a deeper knowledge of the structure and binding properties of these molecules (Graves *et al.,* 2000), which enhances the role of physico-chemical

tools in developing anti-cancer drugs and in understanding their structure–activity relationships. The investigations based on drug–DNA interactions not only help to understand the action mechanisms of some anti-tumor and anti-viral drugs but also to design new DNA-targeted drugs and to screen these drugs in vitro. This emerging field of research has got much importance in recent years (Uil *et al .,* 2003; Russo *et al.,* 2000; Janjua *et al,.* 2009) and a large number of techniques likes gel electrophoresis (Hamdan *et al.,* 1998), footprinting technique (Mauffret *et al.,* 1991), X-ray crystallography (Kennard, 1993), structural modeling (Riahi *et al.,* 2010), spectroscopy and especially fluorescent spectroscopy (Zhang *et al.,* 2010; Kanakis *et al.,* 2009) have been employed to study the interactions of some anticancer drugs with DNA.

In the present paper, the application of the multivariate curve resolution (MCR) method for the analysis of UV-Vis data is proposed and interaction of acridine-spermine conjugate with chicken blood ds-DNA has been investigated via application of spectrophotometric data at different temperatures. This approach is based on the determination of formation constant of drug–DNA complex and thermodynamic parameters which is showed the strength of bonding between acridine-spermine and DNA as well as spontaneity (enthalpy driven process) and compactness of complex. Thus the goal is to calculation of the thermodynamic parameters and equilibrium constants of formation equilibria of drug-DNA at different temperatures.

Materials and methods

Material

Chicken blood DNA extracted in lab was dissolved in autoclaved distilled water (23, 30 and 37 μM) and their concentration was determined spectrophotometrically at 370 nm using molar extinction coefficient, ε_{370} = 4700 cm^{-1} M^{-1}. Acridine-spermine was used without purification (26, 33 and 40μM). Experiments were conducted in 0.1M sodium phosphate buffer solution with pH 7.30.

Apparatus

Absorption spectra were measured on CARY 100 UV–vis Spectrophotometer (Varian) equipped by temperature controller. Conventional quartz cell (10mm×10mm) were used throughout. The cuvettes were treated with repel-silane prior to measurements to avoid dye adsorption. The pH values were measured by a Metrohm 692 furnished with combined calomel Ag/AgCl electrode.

Computer hardware and software

All absorption spectra where digitized at five data points per nanometer in the wavelength 330-460 nm for and transferred to a computer for subsequent analysis by MATLAB (Mathworks, Version 7.8).

Data treatment: Multivariate analysis

Multivariate UV-Vis data were analyzed with the softmodeling MCR procedure to evaluate pure spectra and concentration profiles of spectroscopically active components present in the system from decomposition of experimental data matrix D according to the equation:

$$D = CS^T + E \quad (1)$$

where C and S^T are data matrices containing concentration profiles and pure spectra for each one of these components or conformations present in the experiment. E contains residual noise not explained by the proposed components or conformations in C and S^T. The MCR procedure applied in this work consisted of the following steps (for a more extensive explanation, see references (Tauler *et al.,* 1995):

1. *Data arrangement*: For an experiment monitored by UV-Vis, the recorded spectra were collected in a table or matrix D. The dimensions of this matrix were N_r rows×λ_m columns, where N_r represents the spectra recorded at successive temperature values and λ_m the number of wavelengths measured.

2. Determination of the number of conformations, N: The number of spectroscopically active conformations N was estimated by applying several methods, like singular value decomposition (SVD) or SIMPLISMA

(Tauler *et al.,* 1995).

3. ALS optimization: The ALS optimization procedure is an iterative method used to solve Eq. (1) for the proposed number of conformations N. This iterative process is started with an initial estimation of the pure spectra S_T for each one of the N components or conformations proposed (Tauler *et al.,* 1995).

Concentration profiles C and pure spectra S^T resolved for each conformation in the analysis of individual data matrices may differ from the true ones because of possible unresolved underlying factor analysis ambiguities (Tauler *et al.,* 1995). This means that concentration profiles and pure spectra may be only one solution within a band of feasible solutions that are bounded by the constraints applied in the calculation. Some of these ambiguities are more easily solved by means of the simultaneous MCR analysis of multiple experiments under different conditions. The model used for MCR simultaneous analysis of several equilibrium systems at different total concentrations is described by Eq. (2):

$$\begin{bmatrix} D_{5.15x10^{-5}M} \\ D_{5.75x10^{-5}M} \\ D_{6.35x10^{-5}M} \end{bmatrix} = \begin{bmatrix} C_{5.15x10^{-5}M} \\ C_{5.75x10^{-5}M} \\ C_{6.35x10^{-5}M} \end{bmatrix} S^T + \begin{bmatrix} E_{5.15x10^{-5}M} \\ E_{5.75x10^{-5}M} \\ E_{6.35x10^{-5}M} \end{bmatrix} \quad (2)$$

This simultaneous analysis of several matrices has already been shown to be more powerful than the separate individual analysis, and allows improvement of the resolution of complex experimental data structures. MCR analysis of column-wise augmented data matrices has been shown to give more reliable solutions, eventually removing rotational ambiguities and rank deficiency problems (Tauler *et al.,* 1995).

All MCR calculations were performed using in MATLAB (version 7.8, The Mathworks Inc., Natick, MA, USA) routines, which can be downloaded from the MCR webpage (ASCII files).

Results and discussion

A change in acridine-spermine spectra with added DNA indicates the formation of some type of acridine–DNA complex. Thus the equilibrium established may be:

Acridine + DNA ↔ acridine – DNA (3)

The absorption spectra of the solution, at different total dye concentrations of DNA (16, 18 and 20 μM) and acridine-spermine (20, 22 and 24 μM), were recorded in the wavelength 330-460 nm and temperature 30–70 °C at 5 °C intervals and pH 7.30. The samples absorption spectra are shown in Fig. 1.

Table 1. Equilibrium constant (K) and thermodynamic parameters values at different concentrations.

Concentration	[DNA]=23μM [acridine]=26 μM	[DNA]=30μM [acridine]=33 μM	[DNA]=37μM [acridine]=40 μM
Log K (30°C)	4.68	4.76	4.78
ΔH° (kJ mol^{-1})	-108.0	-103.6	-105.2
ΔS° (J mol^{-1} K^{-1})	-266.8	-262.5	-265.9

The MCR analysis was repeated with three and four components, and with several combinations of constraints, i.e. non-negativity, equality, unimodality and closure. The best (chemically meaningful) results were obtained when only three components were considered, all of them included in the closure constraint, and when the non-negativity constraint was applied to both spectral and concentration profiles. The lack of fit was 3.61% of the experimental data matrix D, which was considered good taking into account the instrumental technique and the large number of matrices simultaneously analyzed, recorded in independent experimental conditions. The resolved concentration profiles (Fig. 2) and spectra profiles (Fig. 3) show the presence of three components.

In the present paper, by utilizing the Vant-Hoff relation (Antonov *et al.,* 1999; Ghasemi *et al.,* 2004), which describes the dependence of equilibrium constant on temperature, thermodynamic parameters of equilibrium have been determined:

$$\ln K = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

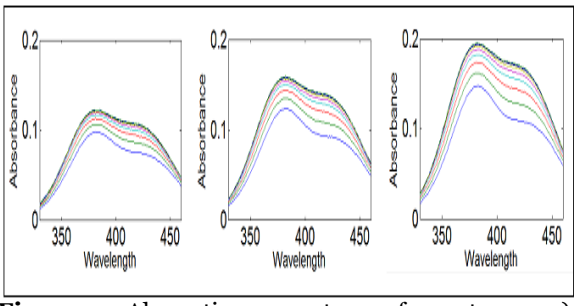

Fig. 1. Absorption spectra of system: a) [DNA]=23µM, [acridine]=26 µM, b) [DNA]=30 µM, [acridine]=33 µM and c) [DNA]=37 µM, [acridine]=40 µM in 5 °C intervals between 30 and 70 °C at pH 7.30.

Where H◦ is the molar enthalpy change, S is the molar entropy change , R = 8.31 $Jmol^{-1}$ K^{-1} the universal gas constant, and T the Kelvin temperature. A linear regression of equilibrium constants with respect to 1/T is then performed, which determines enthalpy and entropy change of the reaction (Table 1). The concentration profiles clearly depicts that as we increase the temperature from 303 to 343 K, the binding constants of all acridine decrease i.e., the association between acridine and DNA weakens as a result of destabilized acridine–DNA complex (Janjua *et al.,* 2009).

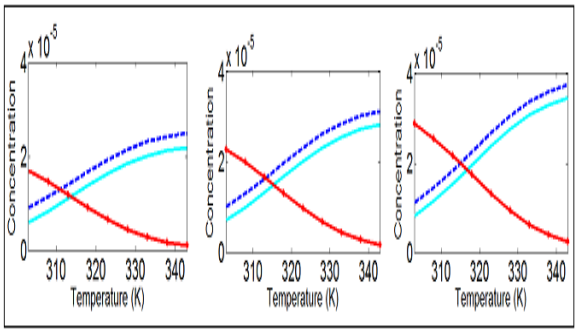

Fig. 2. Concentration profiles of acridine (---), DNA (—) and acridine-DNA (*): a) [DNA]=23µM, [acridine]=26 µM, b) [DNA]=30 µM, [acridine]=33 µM and c) [DNA]=37 µM, [acridine]=40 µM in 5 °C intervals between 30 and 70 °C.

As intercalation is reversible inclusion of drug in between the base pairs of DNA, so at higher temperatures the complex formed between the DNA and acridine molecule may not remain compact as it was at lower temperatures. Increase in temperature changes the geometrical orientation of DNA and the complex formed may become loose and the dissociation may set in. Thus the formation constant values decrease as the temperature is increased. From the negative values of ΔH, one can presume that complex formation is an enthalpy driven process (Shahabadi *et al.,* 2009). During the formation of the complex, the overall entropy of the system also decreases i.e., more compact complex is formed.

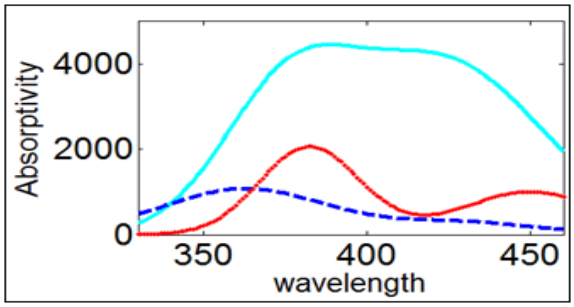

Fig. 3. Spectra profiles of DNA (—), acridine (---) and DNA-acridine (...).

Conclusions

Multivariate analysis has been shown to be a useful tool for the analysis of UV-Vis data recorded during thermal change of equilibria. The application of MCR improved the results obtained by classical univariate analysis of UV-Vis data. The simultaneous analysis of several data matrices, corresponding to different experimental conditions, has allowed the resolution of several systems which are difficult to solve by individual analysis. acridine interacts with ds.DNA in a non-covalent way of interaction via intercalation due to their planarity. The negative values of enthalpy change indicate exothermic of binding of acridine with ds.DNA.

References

Demeunynck M, Charmantray F, Martelli A. 2001. Interest of acridine derivatives in the anticancer chemotherapy. Current Pharmaceutical Design **7,** 1703–1724. http://dx.doi.org/10.2174/1381612013397131

Graves DE, Velea LM. 2000. Intercalative binding of small molecules to nucleic acids. Current organic chemistry **4,** 915–929. http://dx.doi.org/10.2174/1385272003375978

McDonald LA, Eldredge GS, Barrows LR, Ireland CM. 1994. Inhibition of topoisomerase II catalytic activity by pyridoacridine alkaloids from a Cystodytes sp. ascidian: a mechanism for the apparent intercalator-induced inhibition of topoisomerase II. Journal of Medicinal Chemistry **37,** 3819–3827.

Dias N, Jacquemard U, Baldeyrou B, Tardy C, Lansiaux A, Colson P, Tanious F, Wilson WD, Routier S, Mérour JY, Bailly C. 2004. Targeting DNA with novel diphenylcarbazoles. Biochemistry **43,** 15169–15178.
http://dx.doi.org/10.1021/bi0484740

Larsen AK, Escargueil AE, Skladanowski A. 2003. Catalytic topoisomerase II inhibitors in cancer therapy. Pharmacology and Therapeutics **99,** 167–181.
http://dx.doi.org/10.1016/s0163-7258(03)00058-5

Harrison RG, Cuesta J, Chessari G, Read MA, Basra SK, Reszka AP, Morrell J, Gowan SJ, Incles CM, Tanious FA, Wilson WD, Kelland LR, Neidle SA. 2003. Trisubstituted acridine derivatives as potent and selective telomerase inhibitors, Journal of medicinal chemistry **46,** 4463–4476.
http://dx.doi.org/10.1007/s10616-004-5127-z

Uil TG, Haisma HJ, Rots MG. 2003. Therapeutic modulation of endogenous gene function by agents with designed DNA-sequence specificities. Nucleic acids **31,** 6064-6078.
http://dx.doi.org/10.1093/nar/gkg815

Palecek E, Fojta M, Tomschik M, Wang J. 1998. Electrochemical biosensors for DNA hybridization and DNA damage. Biosensors and bioelectrons **13,** 621–628.
http://dx.doi.org/10.1016/S0956-5663(98)00017-7

Oliveira AM, Diculescu VC. 2004. Electrochemical study of quercitin-DNA interactions - part II- in situ sensing with DNA-biosensors. Bioelectrochemistry **64,** 143-150.
http://dx.doi.org/10.1016/j.bioelectrochem.2004.05.002

Johnson MK, Loo G. 2000. Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA. Mutation research **459,** 211–218.
http://dx.doi.org/10.1016/S0921-8777(99)00074-9

Hamdan II, Skellern GG, Waigh RD. 1998. Use of capillary electrophoresis in the study of ligand-DNA interactions. Nucleic acids research**, 26,** 3053–3058.
http://dx.doi.org/10.1093/nar/26.12.3053

Mauffret O, Rene B, Convert O, Monnot M, Lescot E, Fermandjian S. 1991. Drug–DNA interactions: Spectroscopic and footprinting studies of site and sequence specificity of elliptinium. Biopolymers **31,** 1325–1341.
http://dx.doi.org/10.1002/bip.360311110

Kennard O. 1993. Drug–DNA interactions. Pure Applied Chemistry **65,** 1213–1222.
http://dx.doi.org/10.1351/pac199365061213

Riahi S, Eynollahi S, Ganjali MR. 2010. Computational Studies on Effects of MDMA as an Anticancer Drug on DNA. Chemical Biology and Drug Design **76,** 425–432.
http://dx.doi.org/10.1111/j.17470285.2010.01027.x

Zhang GW, Guo JB, Zhao N, Wang J. 2010. Study of interaction between kaempferol–Eu^{3+} complex and DNA with the use of the neutral red dye as a fluorescence probe. Sensors and Actuators, B **144**, 239–246.
http://dx.doi.org/10.1016/j.snb.2009.10.060.

Kanakis CD, Tarantilis PA, Pappas C, Bariyanga J, Tajmir-Riahi HA, Polissiou MG. 2009. An overview of structural features of DNA and RNA complexes with saffron compounds: Models and antioxidant activity. Journal of photochemistry and photobiology, B 95,

204–212.
http://dx.doi.org/10.1016/j.jphotobiol.2009.03.006

Tauler R, Smilde A, Kowalski B. 1995. Selectivity, local rank, three-way data analysis and ambiguity in multivariate curve rsolution. Journal of Chemometrics **9,** 31-58.
http://dx.doi.org/10.1002/cem.1180090105

Antonov L, gergov G, Petrov V, Kubista M, Nygren J. 1999. UV-Vis spectroscopic and chemometric study on the aggregation of ionic dyes in water. Talanta **49,** 99-106.
http://dx.doi.org/10.1016/S00399140(98)003488

Ghasemi J, Niazi A, Westman G, Kubista M. 2004. Thermodynamic characterization of the dimerization equilibrium of an asymmetric dye by spectral titration and chemometric analysis. Talanta **62,** 835- 841.
http://dx.doi.org/10.1016/J-talanta.2003-10.003

Shahabadi N, Kashanian S, Darabi F. 2009. In vitro study of DNA interaction with a water-soluble dinitrogen schiff base. DNA and Cell Biology **28,** 589–596.
http://dx.doi.org/10.1089/dna.2009.0881