Light-absorption spectroscopy of mutagen-DNA complex in presence of competing biologically active compounds

***Ie. Iermak*[1], *A. Woziwodzka*[2], *A. Gwizdek-Wisniewska*[3], *J. Piosik*[2]**

[1]Institute for Radiophysics and Electronics NAS of Ukraine, 12 Acad. Proskura str., Kharkov, 61085, Ukraine
e-mail: evgenia_e@ukr.net
[2]Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland
e-mail: piosik@biotech.ug.gda.pl
[3]Department of Biotechnology, Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, Kladki 24, 80-822 Gdansk, Poland
e-mail: piosik@biotech.ug.gda.pl

Abstract

Mathematical analysis of absorption spectra of biologically active substances is presented. Two models of interactions of mutagen and caffeine with DNA analyzed and obtained binding parameters show good coincidence. Using different models, which take into account formation of various complexes, it is possible to calculate different binding parameters of such triple systems and choose the model describing corresponding system in the best way.

1. Introduction

One of the modern problems in pharmacology and medicine is a prediction of biological efficiency of small molecules (caffeine, theophylline, etc.) in cells and their influence on drugs activity. Light-absorption spectroscopy is one of the most convenient methods to investigate interaction of biologically active small molecules and drugs with nucleic acids. This method can be used to analyze a competitive binding in three – component systems ligand$_1$ – DNA – ligand$_2$ and allows to obtain binding parameters of ligand-polynucleotide complexes in solution.

Theophylline (Tph) and caffeine (CAF) are the most widespread representatives of methylxantines and they are used widely as components of diets and medical drugs [1, 2]. It is also known that Tph and CAF decrease the biological efficiency of several anticancer drugs such as daunorubicin, doxorubicin and mitoxantrone by interaction with aromatic chromophores of these ligands [3]. Direct interaction of methylxantines with medical drugs can be the one reason for decrease of the biological efficiency of several ligands in the presence of Tph and CAF. Another reason can be the competition of methylxantines and DNA-binding ligands for DNA binding sites. In work [4] authors say that Tph and CAF can de-intercalate ethidium bromide (EB) and acridine orange from calf thymus DNA. It is also known that Tph and CAF directly interact with RNA by H-bonds with nucleic bases and Tph binds strongly to RNA than CAF [5]. Previously binding constant of CAF with 5'-d(TGCA) ($K = 246\pm18\ M^{-1}$) [6] and with calf thymus DNA ($K = 190\pm20\ M^{-1}$) was calculated [7].

Mathematical analysis of a mixture containing three components: ligand, CAF and DNA, which interact with each other, is very difficult. A commonly used model developed by McGhee and von Hippel describes ligand-DNA interactions [8]. Another model of ligand-DNA interactions was created by Nechipurenko Yu. D. [9]. Analysis of interactions between ligands with CAF is much more complicated because of the self-association and heteroassociation of CAF and ligands.

In this work we compare two existing models: based on McGhee–von Hippel and Nechipurenko Yu. D. equations for mathematical analysis of a three-component mixture containing light-absorbing ligand (ethidium bromide), CAF and DNA from calf thymus.

2. Mathematical models of ethidium bromide and caffeine interactions with DNA

Calculation of binding parameters of ligand – DNA complexes (binding constants and binding site sizes) have been done according to two different binding models.

In *Model 1* only one type of CAF – DNA and EB – DNA complexes is presumed. Values of ligands binding sites n_1 and n_2 are allowed to vary in a wide region of values. Equilibrium concentrations of free and bound ligands for every mixture with total concentrations of ligands and DNA C_{Di}, C_{pi}, respectively, are calculated according to equations (1)-(4):

978-1-4244-6051-9/11/$26.00 ©2011 IEEE

$$\frac{R_1}{m_1} = K_1 \left[\frac{1 - R_1 \times n_1 - R_2 \times n_2}{1 - R_1 \times n_1 - R_2 \times n_2 + R} \right]^{n_1} \left(1 - R_1 \times n_1 - R_2 \times n_2 + R\right) \quad (1)$$

$$\frac{R_2}{m_2} = K_2 \left[\frac{1 - R_1 \times n_1 - R_2 \times n_2}{1 - R_1 \times n_1 - R_2 \times n_2 + R} \right]^{n_2} \left(1 - R_1 \times n_1 - R_2 \times n_2 + R\right) \quad (2)$$

$$C_{D1} = m_1 + R_1 \times C_P + K_{12} \times m_1 \times m_2 \quad (3)$$

$$C_{D2} = m_2 + R_2 \times C_P + K_{12} \times m_1 \times m_2 + K_D \times m_2^2 \quad (4)$$

Equations (1) and (2) describe the competitive binding of two ligands to DNA [9]. Equations (3) and (4) represent the law of mass balance for ligands.

The following designations have been used in equations (1) – (4): m_1 and m_2 are the equilibrium concentrations of free ligands, where index 1 is used for CAF and index 2 for EB, R_1 and R_2 – are the shares of bound ligands determined as quotient from the division of corresponding complexes concentrations to C_P, K_1, K_2 – are the binding constants for ligands 1 and 2 on binding sites n_1 and n_2 DNA bases per ligand, $R=R_1 + R_2$.

Calculation of the equilibrium composition of mixtures and binding constant values according to the Model 1 is carried out by COMP [10] optimization program. COMP optimization program have been developed as new version of the original DALS program by changing the procedure of equilibrium concentrations calculation. In COMP optimization program the equations (1) - (4) are used in order to calculate the equilibrium concentrations in complexation of ligands (or drugs) with polymeric matrices studies [10]. In this program optimal values of molar extinction coefficients, binding constants and binding site sizes for each type of complex are calculated through minimization of the sum of squares of deviations of experimental absorptions A_{ij}^0 from calculated ones A_{ij}, in wide wavelength and concentration ranges.

The values of absorption A_{ij} are calculated according to the Beer's law:

$$A_{ij} = \sum_k \varepsilon_{jk} \times l \times C_{ki} \quad (5)$$

where ε_{jk} is the molar extinction coefficient of k-th component in j-th wavelength, and c_{ki} is the equilibrium concentration of corresponding component in every i-th mixture, l is the optical path length.

The optimization procedure is terminated when the further iterations of optimized parameters (K_i and ε_{ij}) do not improve the value of optimized function for each model tested. At the end of optimization process the values of both Q and Q_{lim} Hamiltonian factors [11] are calculated:

$$Q = \left\{ \left(\sum_{ij} A_{ij}^{\ o} - A_{ij} \right)^2 / \left(\sum_{ij} A_{ij}^{\ o} \right)^2 \right\}^{1/2} \quad (6)$$

$$Q_{\lim} = \left\{ \left(\sum_{ij} e_{ij}^{\ 2} \right) / \left(\sum_{ij} A_{ij}^{\ o} \right)^2 \right\}^{1/2} \quad (7)$$

where e_{ij} is the deviation of absorbance in the i-th mixture corrected to the 1% error in the total component concentrations and the 0.005 optical unit error in the measurement of absorption. Q and Q_{lim} characterize the correspondence of the binding model to the experimental data. The selected model (and corresponding binding parameters n_1, n_2 and ω) satisfy the experimental absorption data if $Q<Q_{lim}$.

As there was a possibility that CAF could make heteroassociates with EB we have checked their influence on the EB spectrum in the absence of DNA. Heteroassociation of CAF and EB was calculated by equations (8)-(9):

$$EB + EB \Leftrightarrow (EB)_2, \quad K_D \quad (8)$$

$$EB + CAF \Leftrightarrow EB\text{-}CAF, \quad K_{EB\text{-}CAF} \quad (9)$$

in DALS [11] optimization program.

In *Model 2* the intrinsic association constants (K_I) of ligands – DNA interactions were calculated using McGhee–von Hippel model [8], from the equation:

$$\frac{r}{C_A} = K_I (1 - nr) \left[\frac{1 - nr}{1 - (n-1)r} \right]^{n-1} \quad (10)$$

where r is binding density expressed by concentration of bound ligand per concentration of DNA (base pairs) C_B/C_P, n – binding site size, C_A – concentration of free ligand form.

Mixed association constants (K_{AC}) of ligands–CAF interactions were calculated using a thermodynamic model of mixed aggregation of ligands with caffeine [12]. In this paper we used the same notation and definition. There are two types of molecules in the examined system: A (ligand, e.g. EB) and type C (e.g. CAF). Molecules C can form self- and mixed-aggregates, whereas molecules A cannot form self-aggregates.

3. Calculation of binding parameters from absorption spectra

Before studying competitive binding we studied heteroassociation of CAF with EB and EB with DNA. Fig. 1, a shows the absorption spectra of CAF – EB mixtures and Fig. 1, b shows absorption spectra of CAF – EB – DNA mixtures in VIS region. Changes in spectra indicate EB interaction with CAF and DNA. On absorption spectra of CAF – EB mixtures we can see isosbestic point that indicates of one complex formation. Instead on spectra of CAF-EB-DNA mixtures there are no isosbestic points because of minimum two light-absorbing complexes: EB – CAF and EB –DNA.

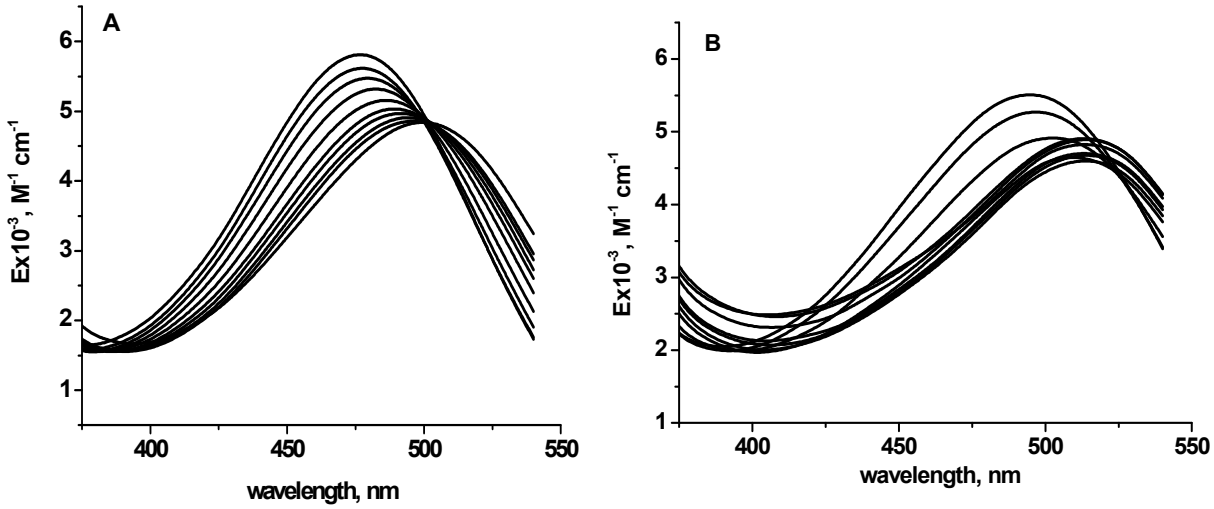

Fig. 1. Absorption spectra of CAF – EB mixtures (C_{EB}=1,3×10^{-4} M, C_{CAF}=0-11.6×10^{-2} M) (panel A). Absorption spectra of CAF – EB – DNA mixtures (C_{EB}=6,9×10^{-5} M, C_{CAF}=5,1×10^{-2} M, C_{DNA}=0-3,2×10^{-3} M) (panel B).

Using absorption spectra measured for EB – CAF, EB – DNA and CAF – EB –DNA mixtures were used to calculate binding parameters of complexes for Model 1 and Model 2. Binding constants calculated for Model 1 are shown in Table 1.

Table 1. Binding constants of EB complexes with CAF and DNA calculated by DALS optimization program and COMP optimization programs for Model 1.

CAF – EB	EB – DNA		CAF – EB – DNA	
K, M^{-1}	K, M^{-1}	n	K_{CAF-EB}, M^{-1}	K_{EB-DNA}, M^{-1}
67.7±2.9	(1.09±0.2)×10^5	2.6	73.4±2.3	(1.04±0.11)×10^5

Binding constants calculated by Model 2 are shown in Table 2.

Table 2. Binding constants of EB complexes with CAF calculated on the basis of Zdunek *et.al*. model [12] and DNA calculated by McGhee–von Hippel equation for Model 2.

CAF – EB	EB – DNA		CAF – EB – DNA	
K, M^{-1}	K, M^{-1}	n	K_{CAF-EB}, M^{-1}	K_{EB-DNA}, M^{-1}
60.2±2.3	(1.4±0.004)×10^5	2.3	96.2±4.9	(1.2±0.04)×10^5

One can see that calculations made by both models give good coincidence of results. Also it can be seen that association constant of CAF – EB complex is approximately 10^3 times less than association constant of EB – DNA complex.

4. Conclusions

We can make a conclusion that caffeine forms heteroassociates with ethidium bromide and also compete with EB for DNA binding sites. As binding constants of CAF with EB and with DNA are much less than binding constant of EB with DNA, the CAF concentration in CAF – EB – DNA mixture should be 100-1000 times higher than EB concentration to see good competition effect. Binding constant of caffeine to nucleic acids is smaller than binding constant of EB to nucleic acids, but this method allows to investigate such subtle effect. This method can be used also to investigate a complexation in any three – component systems even in the case when one of the competitors has a weak affinity to DNA or doesn't absorb neither in VIS, nor in UV region. Different models of binding used for analysis of such triple systems are good enough to describe spectral changes in systems CAF – EB – DNA. Using different models, which take into account formation of various complexes, we can calculate different binding parameters of such triple systems and choose the binding model describing corresponding system in the best way.

5. Literature

1. D.M. Graham, "Caffeine – its identity, dietary sources, intake and biological effects", *Nutr. Rev., V. 36*, 1978, N. 4, pp. 97 – 102.

2. J.F. Williams, S. Lowitt, J.B. Polson, A. Szentivanyi, "Pharmacological and biochemical activities of some monomethylxanthine and methyluric acid derivatives of theophylline and caffeine", *Biochem Pharmacol, V. 27,* 1978, N. 11, pp. 1545 – 1550.

3. M. B. Lyes, I. L. Cameron, "Interactions of the DNA intercalator acridine orange, with itself, with caffeine, and with double stranded DNA", *Bioph. Chem., V. 96*, 2002, P. 53 – 76.

4. I.M. Johnson, S.G. Kumar, R. Malathi, "De-intercalation of ethidium bromide and acridine orange by xanthine derivatives and their modulatory effect on anticancer agents: a study of DNA-directed toxicity enlightened by time correlated single photon counting", *J. Biomol. Struct. Dyn., V. 20*, 2003, N. 5, pp. 677-86.

5. I. M. Johnson, S.G. B. Kumar, R. Malathi, "RNA Binding Efficacy of Theophylline, Theobromine and Caffeine", *Journal of Biomolecular Structure & Dynamics, V. 20*, 2003, N. 5, pp. 687-692.

6. M.P. Evstigneev, K.A. Rybakova, D.B. Davies, "Complexation of norfloxacin with DNA in the presence of caffeine", *Biophys. Chem., V. 121*, 2006, pp. 84 – 95.

7. E. B. Kruglova, Ie. L. Iermak, "Investigation of competitive binding of two ligands: actinocine antibiotic and caffeine to DNA", *Biophysical Bulletin. V. 1*, 2003. N. 12, pp. 64-69 (Russian).

8. J.D. McGhee, P.H. von Hippel, "Theoretical Aspects of DNA-Protein Interactions: Co-operative and Non-co-operative Binding of Large Ligands to a One-dimensional Homogeneous Lattice.", *J. Mol. Biol., V. 86*, 1974, pp. 469-489.

9. Yu. D. Nechipurenko, "Cooperative effects on binding of large ligands to DNA. II. Contact cooperative interactions between bound ligand molecules", *Mol. Biol., V.18*, 1984, pp. 1066-1079.

10. Ie.L. Iermak, O.B. Kruglova, L.H. Palchykovska, I.V. Alexeeva, "Spectrophotometrical study of mechanisms of binding of cytidine analogues and ethidium bromide with DNA", *Biopolymers and cell, V. 23*, 2007, N. 6, pp. 529 – 536.

11. F.R. Harley, C. Burgess, R.M. Alcock, "Solution equilibria", *Ellis Horwood*, London, 1980, 360 p.

12. M. Zdunek, J. Piosik, J. Kapuscinski, "Thermodynamical model of mixed aggregation of ligands with caffeine in aqueous solution, Part II", *Biophys. Chem., V. 84*, 2000, 77-85.