PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2017, **51**(3), p. 171–175

Biology

SPECTRAL STUDY OF MITOXANTRONE WATER-SALINE SOLUTIONS

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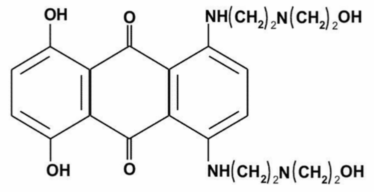
Self-association process of mitoxantrone molecules in water-saline solution depending on concentration, temperature and solution ionic strength has been studied. It was shown that in water-saline solutions associates of mitoxantrone molecules are formed and their domination (stacking-like or molecules are bound by hydrogen bonds) strongly depends on not only mitoxantrone concentration, but also temperature and solution ionic strength.

Keywords: mitoxantrone, self-association, water-saline solution, absorption spectrum.

Introduction. Mitoxantrone (see Scheme) is an anticancer drug that has been successfully used in cancer chemotherapy [1–3]. Like anthracyclines (daynomitsin, adriamycin etc.) mitoxantrone (MTX) binding with DNA changes chromatin structure [4] and DNA compaction [5], moreover it possesses relatively low toxicity compared with anthracyclines, by which preferable application of MTX in clinics is conditioned [3, 6]. The interaction of MTX with double-stranded nucleic acids is spectrophotometrically studied [6–16]; DNA–MTX binding thermodynamic parameters are determined [6, 9, 15, 16]. In [5, 6, 8, 13, 15, 16] the quantitative investigation of MTX–DNA complexes is carried out at relatively high concentrations of MTX, where the formation of self-associates of MTX molecules prevails [9, 17–19]. The modes of self-association of MTX molecules in water-saline solutions were studied in details by NMR method. It was shown that in the process of self-association stack-shaped structures stabilized by associates (mostly dimers) were bound by intermolecular hydrogen bonds and interaction of aromatic rings were formed [19]. In these studies [9, 17–19] the effect of ionic strength and temperature on MTX self-association character was not studied and MTX maximal concentration, when the formation of dimers can be neglected, was not revealed. It should be noted that the formation of the above-mentioned various types of associates changes the absorption spectrum of MTX [17].



Scheme. Structure of MTX.

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In order to reveal how the self-associates influence on DNA–MTX complex formation, the association process of MTX molecules in water-saline solution depending on MTX concentration, temperature and ionic strength has been studied using UV-VIS spectroscopic method.

Materials and Methods. Mitoxantrone ("Sigma", USA), NaCl, Tris ("Serva", Germany) and double-distilled water were used in experiments. MTX concentration was determined spectrophotometrically, using the value of monomeric state extinction coefficient ε_{659}=25090 $M^{-1}\cdot cm^{-1}$ [18]. All preparations were used without further purification. Electronic absorption spectra of MTX were recorded in 400–800 *nm* range on the spectrophotometers Unicam SP8-100 (England) and Cary-219 (USA). Thermostating was realized using a thermostat Haake-F3 (Italy). Studies were carried out in an aqueous solution containing 0.1 *M* NaCl (μ), 0.01 *M* Tris and $5\cdot10^{-4}$ *M* EDTA (pH 7.4). Measurements were carried out in temperature-controlled cells using quartz cuvettes with 10 *mm* volume and tight-fitting stoppers. The accuracy of determining the temperature was ± 0.05°*C* and optical density was 10^{-4} optical units.

Results and Discussion. We consider the association process of MTX molecules with increasing of its concentration at the constant temperature (34°*C*) and μ=0.11 *M* NaCl. Fig. 1 shows the variation of the absorption spectra at increasing of MTX concentration in the solution. As it follows from Fig. 1, with increasing of MTX concentration, the absorption maxima at 665 and 611 *nm* are not shifted the optical density of the solution increases only. Moreover, with increasing concentrations of MTX, a relative increase in absorbance at 665 *nm* is much smaller than 611 *nm*. Consequently, the absorbance at 665 *nm* is likely to characterize the monomeric state of the molecules MTX, and the maximum absorbance at 611 *nm* of associated state. In Fig. 1 the dependence of the optical density at 659 *nm* (A_{659}) on total MTX concentration (C_0) in solution is presented. As it follows from Fig. 2, with C_0 enhancement, A_{659} increases approximately linearly in $0<C_0\leq3\cdot10^{-6}$ *M* interval, and then disrupts linearity that can indirectly indicate the formation of associates. Therefore, μ=0.11 *M* NaCl and temperature of 34°*C* at concentrations greater then $3\cdot10^{-6}$ *M*, association process becomes noticeable and they cannot be neglected.

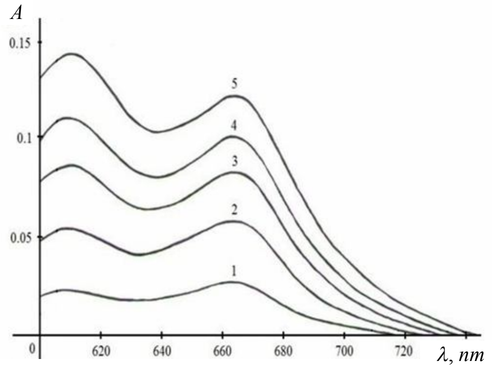

Fig. 1. Absorption spectra of MTX at different C_0, 34°*C* and μ=0.11 *M* NaCl. C_0=$1.0\cdot10^{-6}$*M* (1); $2.6\cdot10^{-6}$ *M* (2); $4.2\cdot10^{-6}$ *M* (3); $7.1\cdot10^{-6}$ *M* (4); $1.2\cdot10^{-5}$ *M* (5).

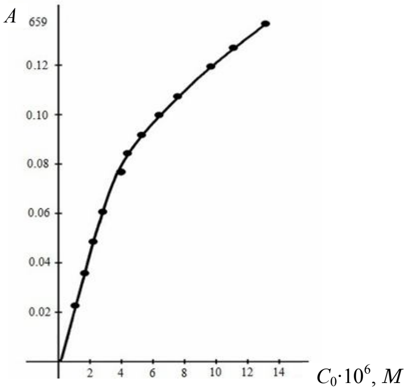

Fig. 2. Absorbance at λ=659 *nm* on MTX concentration in the solution at 34°*C* and ionic strength 0.11 *M* NaCl.

The phenomenon of spontaneous association of MTX molecules in solution is clearly detected from the analysis of the temperature dependence of the absorption spectra in the visible region of the spectrum (Fig. 3). As it follows from Fig. 3, at a constant concentration of MTX (C_0=6.2·10^{-6} *M*) and μ=0.11 *M* NaCl, with an increase in temperature the optical density of the solution increases, wherein the wavelength of maximum absorption is not changed. Simultaneously isosbestic point is observed at 695 *nm*, which is not altered with increasing temperature. The presence of isosbestic point or points indicates that there is a superposition of two independent absorption spectra.

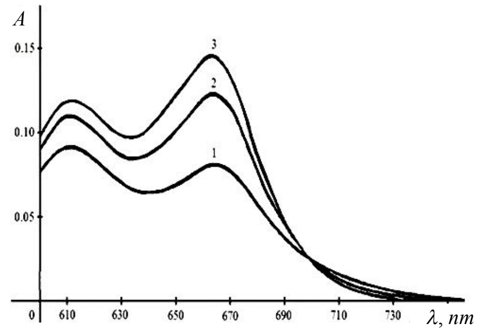

Fig. 3. MTX absorption spectra (C_0=6.2·10^{-6} *M*) at different temperatures at μ=0.11 *M* NaCl : 1 – 28.5°*C*; 2 – 48.5°*C*; 3 – 63.2°*C*.

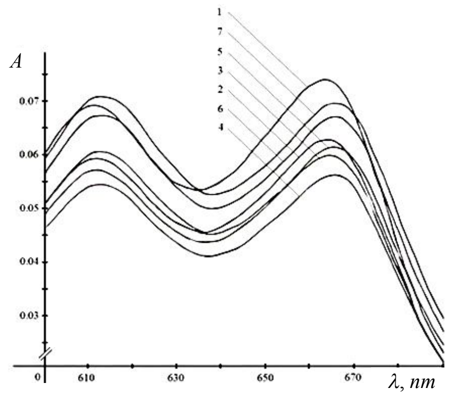

Fig. 4. Change of MTX absorption at ionic strength increasing, 34°*C*. During titration process C_0=3.5·10^{-6} *M* remains constant. Solution ionic strength changes from 5·10^{-3} *M* NaCl (1); 3·10^{-2} *M* (4) to 0.25 *M* (7).

At MTX tested concentrations (C_0=6.2·10^{-6} *M*) in solution there are mainly monomers and dimers. As the temperature increases, the fraction of molecules of MTX-dimers decreases. In [17, 19] it was shown that at the formation of stack-shaped associates, the maximum absorption wavelength was not changed, only the absorption decreased. With high probability, at studied ionic strengths MTX molecules form stack-shaped associates. The presence of an isosbestic point in the absorption spectra (Fig. 3) also indicates two states in the solution. From the absorption spectra shown in Fig. 3 follows that by temperature increasing from 28.5 to 63.2°*C* a relative absorption increase in the absorption maxima grows differently: A_{665} approximately 1.8 times and A_{661} increases 1.3 times. As the temperature increases, at a constant concentration C_0 of MTX in the solution the proportion of the monomers increases, it is possible to argue that the absorbance at 665 *nm* characterizes the state of the monomeric molecules of MTX.

Therefore, MTX molecules in the solution are located in two spectrophotometrically distinct states: monomeric and stack-shaped associated (quite possibly dimerized), the share of the latter decreases with increasing of the temperature in the studied temperature range (from 28.5 to 63.2°*C*).

The association process was also studied, depending on the ionic strength at a constant temperature (34°*C*). At constant C_0=3.5·10^{-6} *M* with increasing ionic strength from 3·10^{-3} to 5·10^{-3} *M* NaCl absorption of MTX solution decreases, and then with further increasing NaCl concentrations (0.25 *M* to) absorbance monotonically increases (Fig. 4). Moreover, when the optical density decreases a red shift is observed on the absorption spectra in the visible region of the spectrum (≈ in 3 *nm*): $\lambda_{\max}$ is shifted from the value of 662 and 610 *nm* to 665 and 613 *nm* respectively. When the optical density begins increasing, the absorption maximum at 665 and 613 *nm* remains unchanged. In [9] the absorption spectra of MTX with increasing polarity of the solvent were investigated. It was shown that a decrease in solvent polarity $\lambda_{\max}$ ($\lambda_{\max}$=660 *nm* for MTX in double-distilled water) is shifted to longer wavelengths due to a decrease in the number of molecules of hydrogen bonds with water molecules and MTX. It should be noted that the red shift is observed upon binding MTX to DNA [6, 9, 15, 18]. Studies have also shown [17] that in aqueous solutions of phenolic OH groups, MTX forms hydrogen bonds with the molecules of polar solvent. At the same time by means of intermolecular hydrogen bonds can form molecular associates MTX [17, 19]. This ionic strength sets the balance between these kinds of associates which depend on the polarity of environment.

Taking into account the results of [17, 19] it can be assumed that an increase in the ionic strength from $3\cdot10^{-2}$ to $5\cdot10^{-3}$ *M* NaCl form two types of associates (stack-shaped and associated with intermolecular hydrogen bonds), which leads to a decrease in the optical density of the solution and red shift. A further increase in ionic strength likely reduces the proportion of stack-shaped associates with almost constant concentration of MTX molecules linked by intermolecular hydrogen bonds (in the field of NaCl concentration the red shift of the characteristic changes in the number of hydrogen bonds is observed).

Therefore, by increasing the ionic strength from $3\cdot10^{-2}$ to $5\cdot10^{-3}$ *M* NaCl a structure formed by associates of MTX molecules should differ from associates formed at higher ionic strengths. Summarizing the dependence of the absorption spectra of the concentration of MTX on the ionic strength and temperature of the solution, it can be assumed that in an almost physiologic conditions, increasing the concentration of MTX and reducing the solution temperature MTX lead to the formation of stack-shaped associates (more likely dimers) and due to an increase of ionic strength changing water structure (polarity protection), which leads most likely to the formation also of longitudinal associates: MTX molecule by means of intermolecular hydrogen bonds forms associates.

Conclusion. From the comparison of experimental data obtained from the spontaneous association of molecules of mitoxantrone, it can be concluded that the aqueous salt solutions, where the interaction of mitoxantrone with nucleic acids is usually studied, almost always associates of molecules of mitoxantrone are formed, the structure of which (stack-shaped or molecules are connected by hydrogen bonds) strongly depends not only on the concentration of mitoxantrone, but also on the temperature and ionic strength. Moreover, if the conditions are close to physiological concentrations of mitoxantrone in the order of $3\cdot10^{-6}$ *M*, almost all molecules of mitoxantrone are in monomeric state, at lower ionic strengths they are at such low concentrations that can form associates. Moreover, the reduction in the ionic strength to 0.03 *M* NaCl concentration leads to increasing shaped stack associates, and further decreasing to 0.005 *M* NaCl, leading to a decrease in the shape of associates of both types (shaped stack and linked by hydrogen bonds).

Received 26.12.2016

REFERENCES

1. **Hagemeister F.** et al. The Role Mitoxantrone in the Treatment of Indolent Lymphomas. // Oncologist, 2005, v. 10, p. 150–159.
2. **Armitage O.J.** The Role of Mitoxantrone in Non-Hodgkin's Lymphoma. // Oncology, 2002, v. 16, p. 490–502.
3. **Sazgarnia A.** et al. *In vitro* Survival of MCF-7 Breast Cancer Cells Following Combined Treatment with Ionizing Radiation and Mitoxantrone-Mediated Photodynamic Therapy. // Photodiag. Photodyn. Therapy, 2013, v. 10, p. 72–78.
4. **Citarella R.V.** et al. Activity of a Novel Anthracenyl Bishydrazone, 9,10-Anthracenedicarboxyaldehyde Bis[(4,5-dihydro-1H-imidazol-2-yl)hydrazone] Dihydrochloride, Against Experimental Tumors in Mice. // Cancer Res., 1982, v. 42, p. 440–444.
5. **Lown J.W.** et al. Interactions of the Antitumor Agents, Mitoxantrone and Bisantrene, with Deoxyribonucleic Acids Studied by Election Microscopy. // Mol. Pharmacol., 1983, v. 25, p. 178–184.
6. **Awashi P.** et al. Multispectroscopic Methods Reveal Different Modes of Interaction of Anti Cancer Drug Mitoxantrone with Poly(dG-dC) and Poly(dA-dT). // J. Photochem. Photobiol. B: Biology, 2013, v. 127, p. 78–87.
7. **Li N.** et al. Interaction of Anticancer Drug Mitoxantrone with DNA Analyzed by Electrochemical and Spectroscopic Methods. // Biophysical Chem., 2005, v. 116, p. 199–205.
8. **Rao M.** et al. Interaction of Antitumor Agent Mitoxantrone with poly[d(G-C)] – a Circular Dichroic Study. // Indian J. Biochem. Biophys., 1989, v. 26, p. 5–8.
9. **Babayan Y.S., Manzini G.** The Interaction of Antitumoral Preparation Mitoxantrone with Double Helical Nucleic Acids. // Molecular Biologia, 1990, v. 24, № 4, p. 1084–1094.
10. **Agarwal Sh.** et al. Spectroscopic Studies of the Effects of Anticancer Drug Mitoxantrone Interaction with Calf Thymus DNA. // J. Photochem. Photobiol. B, 2013, v. 120, p. 177–182.
11. **Tang L.** et al. Study on the Interaction of Anticancer Drug Mitoxantrone with DNA by Fluorescence and Raman Spectroscopies. // Chinese Optics Lett., 2006, v. 4, p. 101–104.
12. **Hajihassan Z.** et al. Interaction of Mitoxantrone, as an Anticancer Drug, with Chromatin Proteins, Core Histones and H1 in Solution. // Inter J. Biol. Macromol., 2011, v. 48, p. 87–92.
13. **Nowiska A.M.** et al. Chromium(VI) but Not Chromium(III) Species Decrease Mitoxantrone Affinity to DNA. // J. Phys. Chem., 2013, v. 117, p. 1021–1030.
14. **Dogra S.** et al. NMR-Based Structure of Anticancer Drug Mitoxantrone Stacked with Terminal Base Pair of DNA Hexamer Sequence d-(ATCGAT)$_2$. // J. Biol. Struc. Dyn., 2014, v. 32, p. 1164–1183.
15. **Dogra S.** et al. Interaction of Anticancer Drug Mitoxantrone with DNA Hexamer Sequence d-(CTCGAG)$_2$ by Absorption, Fluorescence and Circular Dichroism Spectroscopy. // J. Photobiol. B: Biology, 2013, v. 123, p. 48–54.
16. **Bhattacharyya J.** et al. Intercalative Interaction of Anticancer Drug Mitoxantrone with Double Stranded DNA: A Calorimetric Characterization of the Energetics. // J. Chem. Thermodynamics, 2014, v. 75, № 8, p. 45–51.
17. **Lee B.S., Dutta P.K.** Optical Spectroscopic Studies of the Antitumor Drug 1,4-dihydroxy-5,8--bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (Mitoxantrone). // J. Phys. Chem., 1989, v. 93, p. 5665–5672.
18. **Kapuscinski J., Dazzynkiewicz Z.** Interactions of Antitumor Agents Ametantrone and Mitoxantrone (Novantrone) with Double-Stranded DNA. // Biochemical Pharmacology, 1985, v. 34, p. 4203–4213.
19. **Davies D.B.** et al. Self-Association of the Antitumour Agent Novatrone (Mitoxantrone) and Its Hetero-Association with Caffeine. // J. Chem. Soc., Perkin Trans, 2001, v. 2, p. 61–67.