

Solvent type influence on thymidine UV-sensitivity

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Abstract. Thymine is the most sensitive DNA nucleobase to UV-irradiation. In the thymidine solution the photoreactions probability is in dependence on the solvent properties which determine stacking-mediated thymidine association and lifetime of excited states. In this work we investigated the degree of UV-irradiation induced thymidine damages in water, salt (NaCl) and ethanol solvents using UV absorption and circular dichroism (CD) spectrometry, and MS ESI method. In the explored systems thymidine association degree rose in the following solvent order: 1 M NaCl, water, ethanol. UV-absorbance and CD intensity fell in the greater extent in the ethanol and water-salt solutions than in water. So the experiment showed that the association degree of thymidine in the solution does not play a main role in its photosensitivity.

1. Introduction

Thymidine (dT) is the most vulnerable to the direct influence of ultraviolet (UV) irradiation nucleoside in DNA. Namely thymine photoproducts cause mutagenic and cancerogenic effects of UV light on a cell [1]. Thymidine can undergo various types of photochemical modifications, i.e. dimerization (with formation of cyclobutane dimers, 4-6-photoproducts, etc.) and hydration in a DNA sites without secondary structure [1]. These changes in the chemical structure lead to the spectral alternations in the chromophore absorption region which are more manifested in the example of poly-T [2] and frozen thymine solutions [3]. Many researchers indicated the role of monomer association and their mutual orientation in the dimerization process [3-6]. Interaction with solvent plays a significant role in direct and indirect action of radiation on biological macromolecules and their components [7-9]. In the case of UV-irradiation of dT solutions the solvent influences on thymidine photodimerization process via effecting on the monomer association degree, structure of associates and lifetime of excited states [4, 10].

The aim of the present work was to investigate the solvent effects on thymidine self-association and UV-induced dimerization. We studied the UV-absorption and circular dichroism (CD) spectra, and also the mass-spectra with electrospray ionization (MS ESI) of dT in water, ethanol and water-salt solutions before and after UV-irradiation exposure. ESI is relatively soft ionization procedure which does not destroy the analyzed molecules and allows registering complexes formed by ion-dipole and dipole-dipole interactions in a solution [11]. MS ESI is widely used for investigation of nucleoside self-assembling [12, 13] and for identification the DNA photoproducts [14].

2. Experimental section

2.1. Materials

We have used thymidine (Sigma, USA), dissolved in double-distilled water. Thymidine concentration was determined using the known value of its molar extinction coefficient in water $\epsilon(275\text{ nm}) = 8500$



$M^{-1} cm^{-1}$ [15]. We employed sodium chloride NaCl of reagent grade to prepare the saturated solution and ethanol for clinical usage (95% v/v). All solvents were tested by MS ESI and no significant admixtures were revealed. Preparations were carried out with weighting.

2.2. UV-exposure conditions

A low-pressure Hg lamp DRB8 ($\lambda=254nm$) was used as a source of UV-radiation. Dose rate $I=4.14 MJ/(s cm^2)$. UV-exposure of thymidine solutions ($C=10^{-3}-10^{-4}M$) was carried out under the aerobic conditions in a quartz cells with optical path length of 1 cm and surface area $S=4 cm^2$. The adsorbed dose D in J/mol (dT) was determined according to the Lambert–Beer law:

$$D = \frac{I(1 - e^{-A_{254}})St}{V_{dT}},$$

where A_{254} is the optical density of the irradiated solution at $\lambda=254 nm$, t – time of irradiation (sec), V_{dT} is the amount of substance of thymidine (dT) in the irradiated sample (mol). Hence, from the expression one can see that the calculated doses adsorbed by the solutions were corrected. This could be achieved through permanent measurement of solutions absorption spectra during the irradiation what allows considering the changes in the solutions absorption.

2.3. Methods

UV absorption spectra of solutions were obtained by spectrophotometer SF-56 (Russia), CD spectra were measured by autodichrograph Mark V (Jobin Ivon, France), the examined parameter is the difference in molar extinction coefficients of nucleoside solution $\Delta\varepsilon=f(\lambda)$ for right and left polarized light. MS study was performed by «MaXis» (Bruker Daltonik GmbH, Germany) using ESI-QTOF analysis, in the positive ions mode. To promote the positive ions formation the solution for MS were prepared at $pH(3.0\pm 0.1)$ by adding HCl.

3. Results and discussions.

Solvent sufficiently influenced spectral properties of thymidine. Its molar extinction coefficient obtained in the different medium under investigation is given in the table 1. It should be noted that the shape of thymidine UV absorption spectrum and its maximum position (around 267 nm) did not alter in water-salt or ethanol solutions as well as in water solution (figure 1).

Table 1. Thymidine molar extinction coefficient in the different solvents.

Solvent	$\varepsilon (\lambda_{max}=268 nm)\pm 50, M^{-1}cm^{-1}$
water	9560
ethanol 84% v/v	7860
1 M NaCl	9740
3.6 M NaCl	10200
5M NaCl	10700

CD spectra of unirradiated solutions are shown in figure 2a. Summarizing the observed spectral alterations of dT due to the solvent change one can suggest the following. It is known that in water solution nucleosides have the tendency to stacking associates formation [16, 17]. The electrolyte concentration increase leads to the demolition of these associates or changes in their mutual orientation. It was revealed from the growing hyperchromic effect in dT UV absorption spectra with the rising in ionic strength of solutions (see table 1, figure 1). On the contrary, in ethanol dT solutions we could see the hypochromic effect in comparison with water medium (table 1, figure 1) and also the considerable monotonous the CD bands intensity upward growth (especially the positive band, which

rocketed from $\Delta\varepsilon(274)=1.4$ in water to $\Delta\varepsilon(274)=4.6$ in ethanol 84% v/v) attending to ethanol concentration increase. It can be caused by stronger thymidine molecules stacking association in ethanol than in water (this conclusion is corroborated by the data of nucleosides solubility in water and ethanol [18]), or by changes in the dT orientation in the associate.

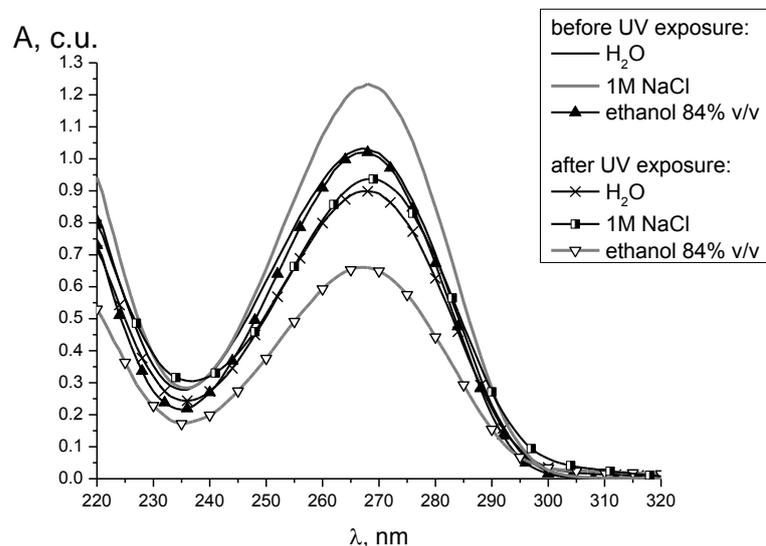


Figure 1. UV absorption spectra of thymidine solutions, conditions and type of solvent are indicated on the graph. Thymidine concentrations in water $1.08 \cdot 10^{-4} \text{M}$, in 1M NaCl $1.06 \cdot 10^{-4} \text{M}$, in ethanol $1.3 \cdot 10^{-4} \text{M}$. The absorbed dose for these solutions irradiated for approximately 5h: $D(\text{water}) = 6.0 \cdot 10^8 \text{ J/mol (dT)}$, $D(1\text{M NaCl}) = 4.2 \cdot 10^8 \text{ J/mol (dT)}$, $D(1\text{M NaCl}) = 3.6 \cdot 10^8 \text{ J/mol (dT)}$.

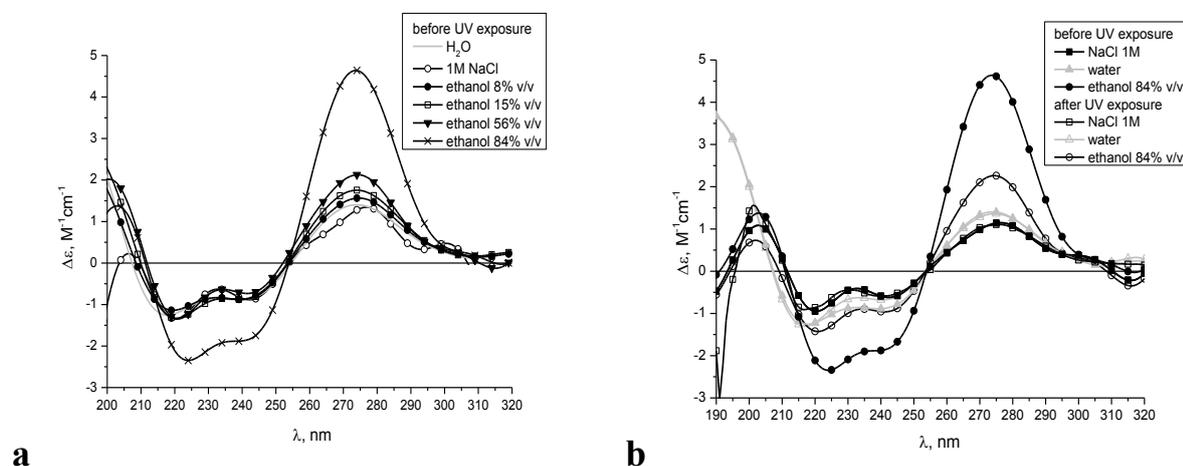


Figure 2. CD spectra of thymidine solutions, conditions and type of solvent are indicated on the graph. Thymidine concentrations and absorbed doses are as in Figure 1. (a) CD spectra of solutions before UV-irradiation. All range of ethanol concentrations (points are 8, 15, 56, 84% v/v) is represented. (b) CD spectra of solutions before and after UV-irradiation. There are demonstrated 1M NaCl, water, and 84% v/v ethanol solutions only.

The MS results confirmed the supposition about the rise in dT association (or aggregation) with the increase in ethanol concentration. In Figure 3 the examples of MS ESI data are presented, all the obtained MS results are given in table 2. The intensity of MS peaks assigned to the various dT

associates mainly larger in ethanol than in water: eight, and two and a half as much in unirradiated, and irradiated solutions, respectively. However, one should be careful in comparing MS data acquired in different solvents: in the medium with smaller dielectric constant (ethanol) the molecular ionization is more preferable. At high electrolyte concentration the mass spectrometrically detected analyte ion intensity decrease sharply [19], so we did not obtain the reliable MS data for dT solutions in 1M NaCl.

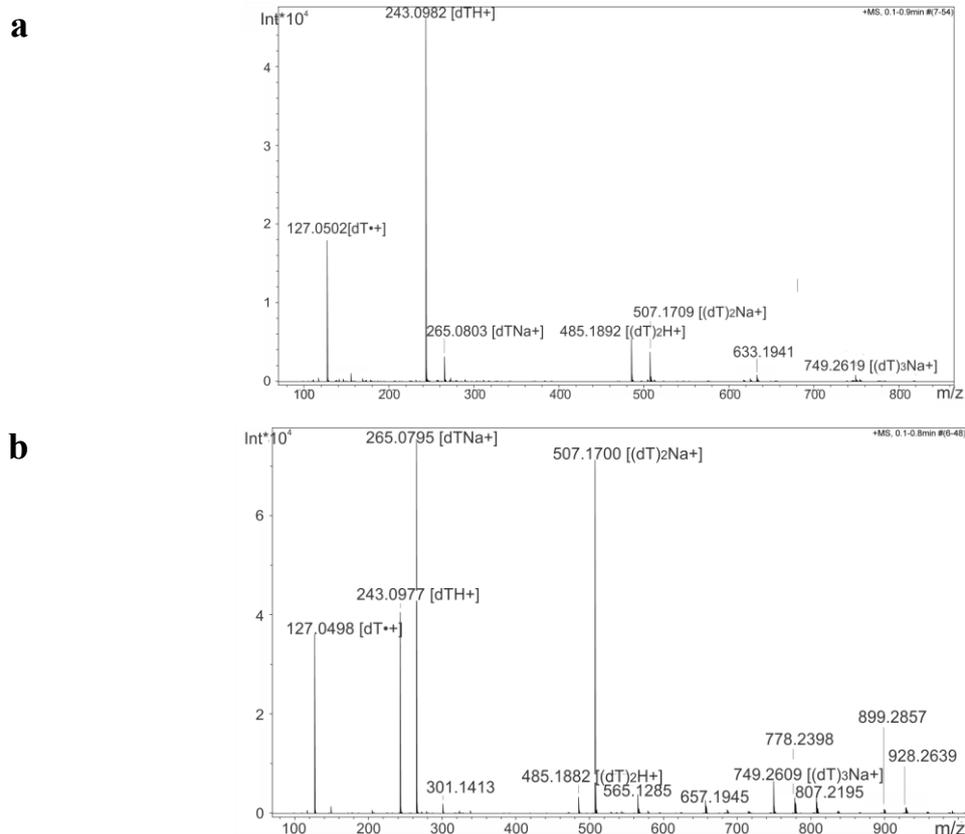


Figure 3. Mass-spectra of dT in water (a) and ethanol 84 % v/v (b) solution before UV-irradiation.

Table 2. The main identified peaks in mass-spectra of thymidine solutions

m/z	ion	water				ethanol 84% v/v			
		before UV-exposure		after UV-exposure		before UV-exposure		after UV-exposure	
		I	I/I(243),%	I	I/I(243),%	I	I/I(243),%	I	I/I(243),%
265.08	dTNa+	3160	7	1000	1	75700	185	28000	34
243.09	dTH+	45800	100	87000	100	40900	100	82500	100
485.19	(dT) ₂ H+	5900	13	15000	17	3040	7	10000	12
507.17	(dT) ₂ Na+	3700	8	1000	1	71300	174	29000	35
749.26	(dT) ₃ Na+	500	1	-	-	6100	15	3000	4
127.05	T•+	17900	39	40000	46	36500	89	50000	61
625.21	(dT) ₅ K+H+	-		-		-		730	1
149.02	TNa+			-		-		2000	2
sum of associates peaks intensities		10100		16000		80440		42730	

The absorbance and CD intensity of dT solutions decreased monotonously with the UV-irradiation dose rise (see figure 1, 2b, 4), at that UV-irradiation affected dT spectral properties in larger extent in ethanol and water-salt solutions than in water. Photodimers formation should lead to the decrease in UV absorbance at 268 nm and rise in absorbance at shorter wavelengths [2]. Note, that the number of dT associates, according to the MS data, after the UV-exposure increased in water (by about 58%) but declined in ethanol (by about 47%) (table 2). This result is unexpected because the probability of photodimerization rises with the increase in association degree of dT in a solution. It is possible that the decrease in solvent polarity leads to a fall in quantum yield of thymidine photodimerization, maybe through decrease in lifetime of dT excited states, or by rearrangement of dT in an aggregate to the less proper for the photodimerization process orientation. It should be mentioned also that there is the information about chromophore destruction under the action of high doses (about $1\text{W}/\text{m}^2$) of UV-irradiation [20], so at the conditions of our experiment we cannot exclude this process.

Consequently our results indicated that the association degree of thymidine in a solution did not play a main role in its photosensitivity. We can assume that photoprocesses in dT solutions depend on the thymidine-solvent interaction causes the structure of dT associates and the lifetime of dT excited states.

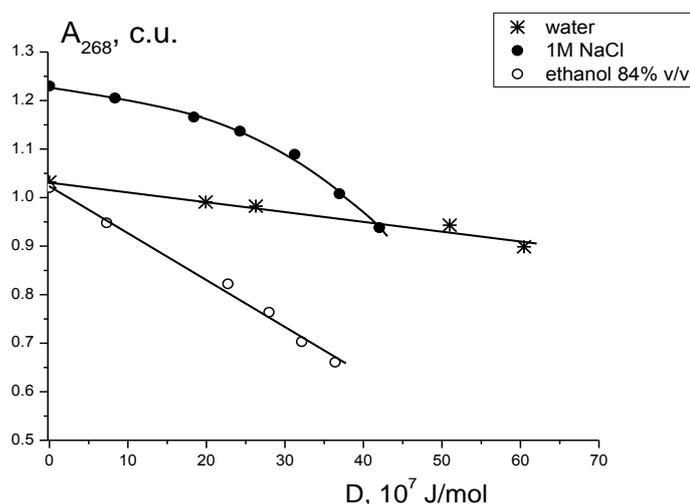


Figure 4. UV-radiation dose dependence of thymidine solutions absorbance intensity in maximum of the spectrum

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