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TOMASZ LESZCZYŃSKI
HENRYK DUŃSKI
Institute of General Food Chemistry
Technical University of Łódź

STUDIES OF INTERACTION BETWEEN (m-AMSA) AND DNA BY VISCOMETRIC AND MELTING PROFILES METHODS

Review: **prof. dr hab. Stanisław Wysocki**

We have examined the interactions between m-AMSA and CT-DNA, using melting profiles and viscometric techniques methods. We observed the increase of thermal stability of DNA after binding m-AMSA, depending on the concentration of the ligand in the solution. The maximal increase of the melting temperature of the m-AMSA/DNA system in comparison to pure DNA, was 6°C for the concentration of m-AMSA to DNA of 0,5. Viscometric examination of interaction of m-AMSA with DNA unambiguously indicate intercalation type of bounds. In the saturation state, 2,76 base pairs occur for one ligand molecule.

1. Introduction

For many years, the nature of the interactions of small molecules to DNA has been a concern of not only physical chemistry, but mainly medical chemistry. Considering the major role of DNA in the regulation of biochemical processes, compounds capable of interacting with it, exhibit a wide spectrum of biological activity, especially the antitumor one.

The m-AMSA itself was, synthesized by Cane in early 70s [1], and belongs to acridine derivatives class. It is characterized by wide bacteriostatic and cytostatic action, and is a leukemia cure [[2]]. Despite of years lasting research, its interactions with DNA, especially at low concentration, is not well known.

Small molecules can interact with DNA in a variety of ways such as: surface binding to their minor or major grooves, intercalation between adjacent base pairs, covalent attachments to the double helix, or electrostatic binding.

Molecular interaction of the drug with DNA may have a great importance to its biological activity. For instance, of the two isomers: o-AMSA and m-AMSA, the first has a higher DNA association constant, but only the second has a strong antitumour effect. In the case of m-AMSA, this cytotoxic effect is due to favorable arrangement of methoxy group, which allows creation of a complex with topoisomerase II and its inhibition [3], [4].

In this paper, type and strength of m-AMSA to DNA binding was examined using viscometric and melting profiles techniques. These activities were essential part of wider research over the mechanism of interactions of acridine derivatives (especially m-AMSA) and DNA. The research were conducted by our Biophysical Chemistry Team and allowed to define the type of binding and size exclusion parameters of this ligand.

2. Experimental details

2.1. Materials

The calf thymus DNA (CT-DNA) and 4'-(9-acridinylamino) methanesulfon-m-anisidide (m-AMSA) produced by Sigma and ethidium bromide (EB) produced by Merck were used in the experiment.

The DNA was dissolved in 0,01 M phosphate buffer (pH 7,0) containing 0,001 M EDTA. Millipore deionized water was used for making aqueous solution. Other reagents were at least analytical grade and were used without further purification.

2.2. Methods

The concentration of both dyes and DNA was calculated on the basis of measured absorbance of the solution, using $\varepsilon_{260} = 12824\ M^{-1}cm^{-1}$ [5] for DNA, $\varepsilon_{434} = 1200\ M^{-1}cm^{-1}$ [6] and $\varepsilon_{480} = 5600\ M^{-1}cm^{-1}$ [7] for m-AMSA and EB respectively. Spectrometric measurements were conducted with BECKMAN DU 7500 spectrophotometer.

Sonificated DNA solutions and freshly made m-AMSA and EB solutions were used. The ligands were prepared from more concentrated DMSO solutions, due to its weak solubility in water [8].

The melting temperature measurements employed spectrophotometric methods [6]. The meter was: Varian Cary 5E spectrophotometer, equipped with thermostated measuring system.

The sample of DNA (typical concentration $5x10^{-5}$ M base pairs) with dye and phosphate buffer as reference material was heated till temperature stabilization and absorbance was measured.

The absorbance measurement was conducted at wavelength of λ = 260nm for DNA and occasionally at λ = 434nm for m-AMSA. The temperature range was 300-360K, at the interval of 2°C.

The viscosity measurements were conducted using Ubblehode viscometer, submerged in a water bath, maintained at 25,0 ± 0,1oC. Flow times were measured 3 times, with a stopwatch of accuracy of ± 0,1s, and the average time over all replicates was recorded. Viscometric titrations were conducted according to Wilson's procedure [9].

Sonificated DNA solution, at $2x10^{-4}$ M base pairs concentration, was placed in viscosimeter. Small volume (0,05-0,1 ml), of concentrated solution of EB or m-AMSA in DMSO of $1,28x10^{-3}$ M and $2,25x10^{-3}$ M respectively, were directly added and flow times were measured after each addition.

The relative solution viscosity was calculated with formula (1)

$$\eta/\eta_0 = (t-t_0)/(t_{DNA}-t_0) \quad (1)$$

where t_0 and t_{DNA} – flow times buffer and buffer containing DNA, t – flow times of buffer containing DNA after ligand addition.

The helix unwinding angle of DNA – Φ, induced by binding m-AMSA was determined by measured flow time of reference standard EB. This compound has well documented unwinding angle of 26° [6], [10], [11].

The Φ induced by binding m-AMSA can be determined using the following relationship [12]:

$$\Phi_E * \nu_E = \Phi_m * \nu_m \quad (2)$$

where: Φ_E and ν_E reflect the ethidium-induced helix unwinding angle and critical saturation ratios, respectively, while Φ_m and ν_m are the corresponding m-AMSA parameters.

3. Results and discussion

3.1. Thermal melting studies of binding between m-AMSA and DNA

Fig. 1 presents graphical method of determination of the melting temperature (T_M). Since all examined profiles were almost linear in the melting region, T_M could be determined as the average of starting and final temperatures of melting process.

In most of the measurements, melting process was traced by estimation of DNA absorbance (at λ=260nm). Example curves are presented at Fig. 2 and all the results of the measurements are compiled in Tab. 1.

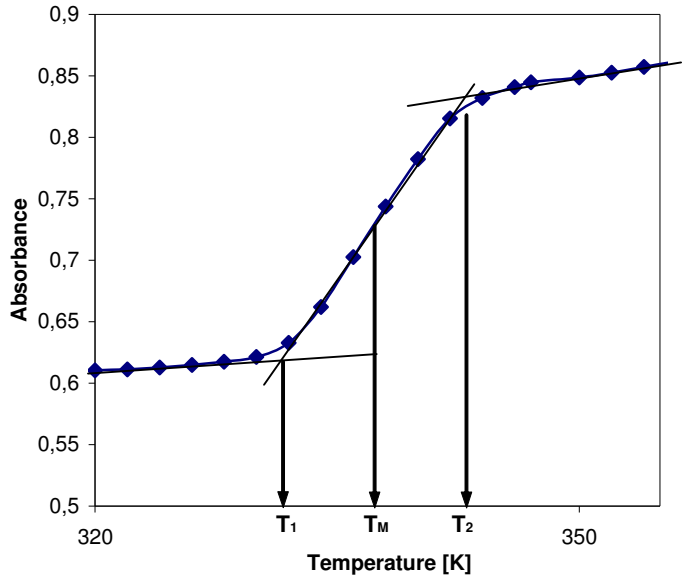

Fig. 1. Graphical method determining the melting temperature T_M. T_1 and T_2 are the starting and final temperatures of melting process

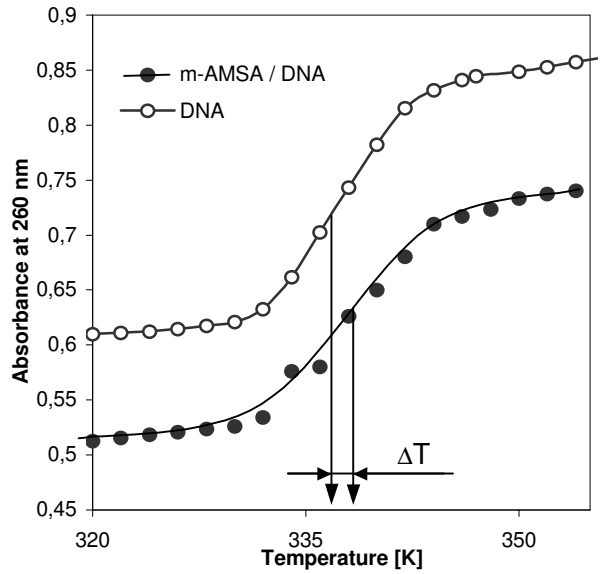

Fig. 2. UV melting profiles of DNA with m-AMSA at r = 0,1 where r is the total ligand to base pair ratio

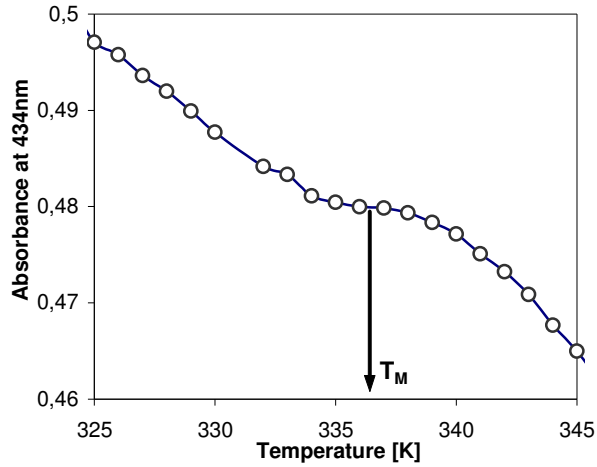

Fig. 3. The absorbance of the solution of DNA and m-AMSA (r = 0,2) with respect to heating temperature

Table 1

Melting temperature T_M DNA samples

Samples	DNA melting temperatures [^oC]	ΔT_M [^oC]
DNA	63,5 ± 0,5	-
m-AMSA / DNA = 0,1	65 ± 0,5	1,5
m-AMSA / DNA = 0,5	69,5 ± 0,5	6
m-AMSA / DNA = 0,2 (experimental conditions in Fig.3)	63,5 ± 0,5	0

In our experiment we observed a repeatable melting temperature of 63,5 ± 0,1^{o}C. The result is close to values obtained by Gomley [13] and Sissi [14], which were 65 ± 1,6^{o}C and 65,6^{o}C, but differs from some other results (58,5^{o}C presented in [15]). Observed differences of T_M of CT-DNA, derive from varying experiment conditions, in particular ionic strength and buffer pH.

Adding m-AMSA to the solution results in DNA thermal stability increase. This leads to rise of ΔT_M parameter, depending on the m-AMSA concentration in the solution. For example increasing r = 0,1 to 0,5, causes a rise of ΔT_M from 1^{o}C to 6^{o}C. These results are in perfect agreement with Antonini [6] and Bailly [16], who achieved the rise of ΔT_M from 1^{o}C to 5^{o}C, for the same r value as ours.

The rise of m-AMSA concentration in the solution, increases the amount of molecules reacting with DNA and its thermal stability. A question arises, what is the mechanism of interaction between the ligand and DNA, and whether its dissociation accompanies the melting process. The last we tried to answer, observing the amount of dissociating ligand during DNA melting process. In that, we took advantage of the fact that molar extinction coefficients of

m-AMSA free and bound to DNA differs significantly [17], [18]. The results of the experiment are presented in Fig. 3. A surprising effect of gradual decrease of absorbance at temperature rise, may be observed. The effect is caused by low thermal stability of m-AMSA, exposed to long term (c.a. 3 hours) heating. The mentioned low thermal ligand stability is corroborated by observed changes in the shape and intensity of the spectrum of m-AMSA bound with DNA, at room temperature close to T_M [these results will be published].

The determined value of T_M (Fig. 3) is the same as in the solution containing only DNA. Moreover, the amount of ligand dissociated from DNA, in the melting process, is small. The results, suggest that most of the bound m-AMSA dissociates before the melting process is initiated and the rest is bound so strongly that it moves the process towards higher temperatures. It is also possible that only one of the two types of m-AMSA with DNA bound dissociates [these results will be published] or the behavior is due to heterorganic structure of DNA [18].

3.2. Determining the type of binding with viscometric titrations

The analysis of the shapes of viscometric titrations curves is a convenient way to determine the type of binding of ligand with DNA [19], [20]. Fig. 4 and 5 present relative viscosity (η/η_0) versus ligand to DNA ratio (r), during the titrations, for m-AMSA and EB. The last is a typical intercalator. In both cases, one may observe nearly linear increase of relative viscosity, collapsing at approximately ~1,95 for m-AMSA and ~1,6 for EB. Above these points the η/η_0 rise is less dynamical.

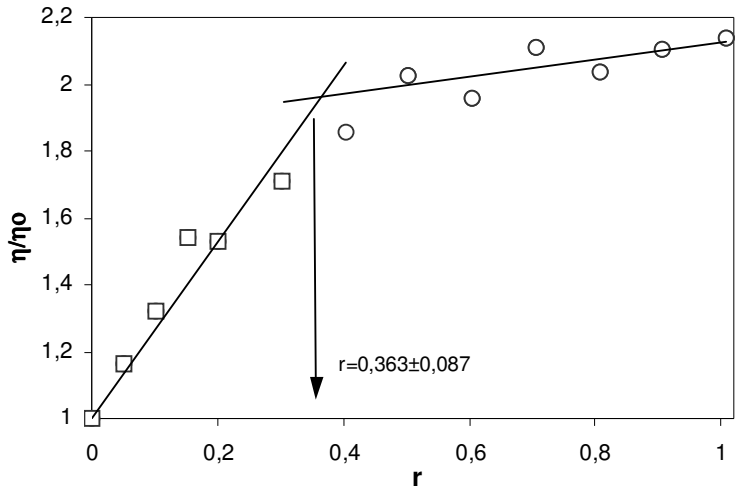

Fig. 4. Viscometric titrations of DNA with m-AMSA;
r_s is the critical saturation ratio

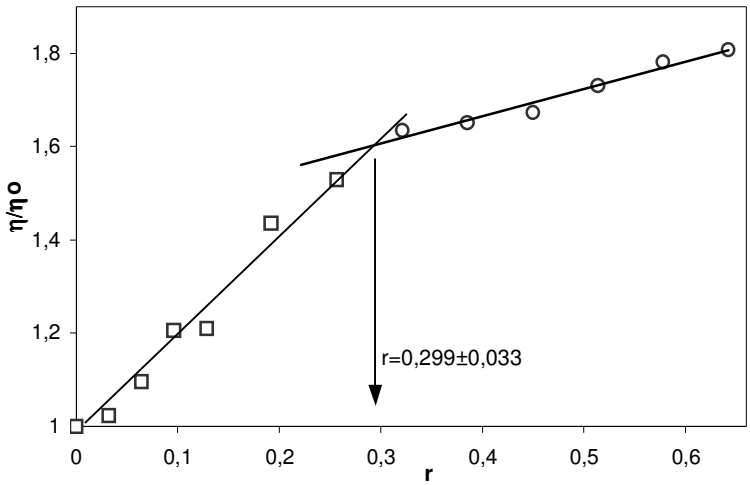

Fig. 5. Viscometric titrations of DNA with EB

These result lead to the following conclusions:

- in both cases collapse of relative viscosity occurs, at values characteristic for intercalation process ($\eta/\eta_0 = 1,9 \pm 0,3$ [20])
- collapse point is strictly related to critical saturation ratio r_s being the maximal amount of intercalating ligand molecules relative to one base pair of DNA

We obtained $r_s = 0,363 \pm 0,087$ for m-AMSA and $r_s = 0,299 \pm 0,033$ for EB (Fig. 4 and 5).

The reciprocal of r_s is, so called, size exclusion parameter ω, useful for describing the binding isotherms. The value of this parameter for m-AMSA was calculated to be $2,76 \pm 0,66$, which is convergent with the results of Rebecca [18]. Simultaneously, the value was $3,34 \pm 0,37$ for EB, which is close to the outcome we achieved during the fluorescent measurements ($\omega = 2,86 \pm 0,12$ [these results will be published]).

Using the equation 2 with the values of r_s, unwinding angle was determined to be $21,5^o \pm 3,8^o$ for m-AMSA. The angle value is close to 21^o found by S.Neidle [21], Denny [22] and $20,5^o$ in [23] or 20^o in [24].

Typical unwinding angle of DNA in β form is 36^o. Intercalation of the ligand leads to its deterioration to 10^o-26^o [25], which is characteristic for this process. The value of $21,5^o$, achieved by us, stays within the range characteristic for intercalators and confirms this type of binding of m-AMSA with DNA.

As a result of intercalation the first and the second structure of DNA stays intact. Unwinding due to a slipped ligand is a reason for the change of tertiary DNA structure. The DNA molecule is partly elongated and the distance between two neighboring base pairs increases by 3,4Å to 7-8Å on average [26].

The increase in thermal stability of DNA after binding with m-AMSA, manifesting as the rise of melting temperature of double DNA helix, is considered to be a proof for intercalation as well [27].

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BADANIA ODDZIAŁYWAŃ POMIĘDZY m-AMSA A DNA METODAMI WISKOZYMETRYCZNYMI ORAZ PROFILI TOPNIENIA DNA

Streszczenie

W pracy badano oddziaływania pomiędzy m-AMSA i CT-DNA metodami wiskozymetrycznymi oraz profili topnienia DNA. Zaobserwowano wzrost stabilności termicznej DNA po związaniu m-AMSA, która zależała od stosunku stężeń m-AMSA/DNA. Największy wzrost temperatury topnienia badanego układu względem roztworu DNA wyniósł 6^{o}C dla stosunku m-AMSA/DNA = 0,5. Pomiary wiskozymetryczne wskazały jednoznacznie na interkalacyjny sposób wiązania m-AMSA z DNA. W stanie wysycenia jedna cząsteczka m-AMSA przypadała średnio na 2,76 par zasad DNA.

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