

INVESTIGATION OF AMITOZYN1, MODIFIED BY
TRIETHYLENETHIOPHOSPHORAMIDE OF *CHELIDONIUM MAJUS*
ALKALOIDS, AND THYMUS DNA INTERACTION AT THE PRESENCE OF
STAINED BIOLOGICALLY ACTIVE MARKERS

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Amitozyn – is an antitumor preparation, obtained from cumulative celandine alkaloids (chelidonin, protopin, berberin, sanguinarin and others) alkylation by triethylenethiophosphoramide (thiotheph). Having strong fluorescence, amitozyn and its particular alkylated components are successfully used for studies of molecular-cellular mechanisms of the tumor process diagnostics and for the control of neoplasms treatment efficiency [1]. “Lighting” capability is also successfully used for studying the interaction of the mentioned above preparations with DNA and RNA [2].

In the current work we presented the research results of amitozyn1 (Am1) binding with thymus DNA using other methods, in particular, UV-spectroscopy and competitive binding methods.

For amitozyn obtaining, *Chelidonium majus* alkaloids were extracted from the mentioned herb and alkylated according to modified method, given in monographs [3]. The obtained preparation was a hygroscopic mass; ethanol was used to dry it. A portion of the preparation transformed to the alcoholic solution called amitozyn1 (Am1). The value of Am1 extinction molar coefficient was found using gravimetric method and the maximum absorption was $\varepsilon_{290} = 7.12 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. To study the competitive binding of two ligands with DNA, well-known intercalator ethidium bromide (EB) and actinomycin D analog, antibiotic of ActII actinocyn line with two methylene groups in side amine chains were used as the

stained markers. At the determination of EB and ActII concentrations, the values $\epsilon_{480} = 5.85 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ [4] and $\epsilon_{400} = 1.61 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ [5] were used. In the current work we used commercial thymus DNA of the calf of “Serva” firm. At the determination of DNA concentration in phosphates moles (C_P^0) the value of molar coefficient of extinction in absorption maximum $\epsilon_{260} = 6.4 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ was used, and values P/D were evaluated as a ratio of the general DNA concentrations (C_P^0) to the concentration of investigated ligand (C_D^0). Spectrophotometric measurements were conducted in the quartz cuvettes with 10 mm optical path length using Specord M40 (Germany) spectrophotometer. The researches of complex formation were conducted in phosphate ($2.5 \times 10^{-2} \text{ M KH}_2\text{PO}_4$, $2.5 \times 10^{-2} \text{ M Na}_2\text{HPO}_4$, pH=6.86) buffer solution.

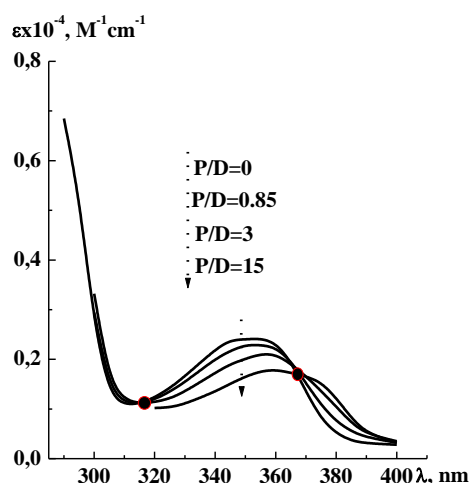


Fig. 1. The absorption spectra of DNA – Am1, $C_{Am1} = 2.04 \times 10^{-4} \text{ M}$ composites in phosphate buffer solution at the different P/D values. Circles illustrate the isobathic points.

As may be seen from the figure 1, in the range of 0 to 15 P/D values Am1 forms with DNA only one complex type, because in the mentioned P/D area the absorption spectra of composites go through the same isobathic points.

To determine complex type, formed by Am1 with DNA, the competitive Am1-DNA-binding was conducted at the presence of well-known intercalator ethidium bromide (EB) [4] and actinomycin D analog, antibiotic of ActII actinocyn line. As Am1 weakly absorbs in the visible spectrum, in contrast to EB and ActII, we conducted the comparative titration of ActII – DNA composites at Am1 and ethidium bromide presence.

EB itself binds to DNA with forming the only one complex type – through intercalation [4] (fig. 2, a). ActII, as may be seen from the fig. 2,b, binds with DNA, forming at least two components that absorb in different ways. This happens because of isobathic point, appearing in the low P/D values area ($\lambda=465$ nm), disappears during the growth of DNA concentration. Further P/D increasing leads to absorption growth and band displacement to the long-wave area ($\lambda_{\max}=465$ nm). According to our data, at the average P/D values ActII binds with DNA in the furrow, and at the high values – intercalates in DNA [5].

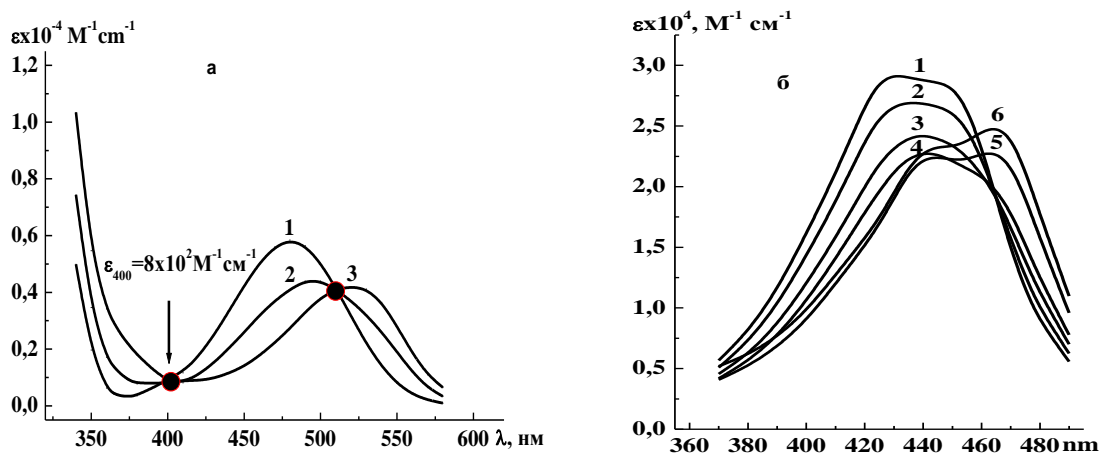


Fig. 2. The absorption spectra of DNA – EB composites at different P/D values: 1-P/D=0; 2-P/D=2; 3-P/D=30, $C_{EB}=1.52 \times 10^{-4}$ M (a); the absorption spectra of ActII – DNA composites at $C_{ActII}=2.79 \times 10^{-5}$ M in phosphate buffer solution at different P/D values: 1 – P/D=0; 2 – 1.6; 3 – 3.6; 4 – 5.7; 5 – 18.5; 6 – 67.5 (b).

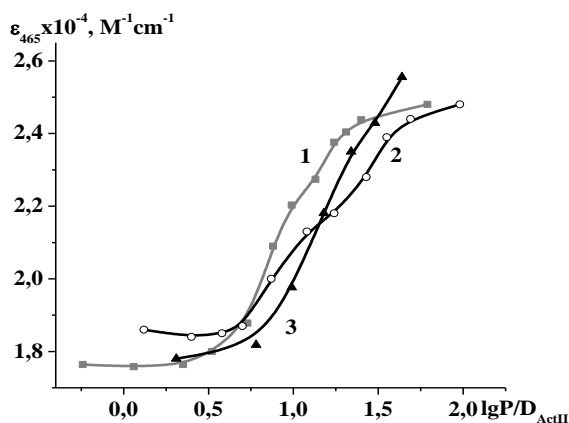


Fig. 3. The titration curves of ActII – DNA composites, $C_{ActII}=4.32 \times 10^{-5}$ M (1), ActII – DNA composites at EB presence ($C_{ActII}=1.71 \times 10^{-5}$ M, $C_{EB}=3.86 \times 10^{-4}$ M) (2), at AmI presence ($C_{ActII}=1.34 \times 10^{-5}$ M, $C_{AmI}=6.59 \times 10^{-5}$ M) (3).

As may be seen from the titration curves of ActII – DNA composites in EB and Am1 presence (fig. 3), EB effects on ActII binding with DNA generally at the high P/D values at the same time preventing ActII intercalation. In contrast to EB, Am1 effects distinctly at the average P/D values, from this it may be concluded that Am1 prevents ActII from binding with DNA in the furrow and, therefore, Am1 itself also forms complexes on the same type of external binding and it does not intercalate in DNA.

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