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INTERACTION OF ERGOSINE AND ERGOSININE METHANESULPHONATES WITH CALF THYMUS DEOXYRIBONUCLEIC ACID IN VITRO

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Interaction of ergosine methanesulphonate (EmS) and ergosinine methanesulphonate (EnmS) with double-stranded and denatured calf thymus DNA was studied under in vitro conditions.
Changes in absorption spectra of both ergot alkaloid derivatives after addition of DNA, together with the data of spectrophotometric titrations of these substances with DNA and an increase in thermal DNA stability in the presence of these compounds pointed to the precisely determined reactions. Intrinsic association constants were 1.6×10^5 M^{-1} and 1.4×10^5 M^{-1}, while the number of binding sites per 100 moles of nucleotides was 4 for both EmS and EnmS. Heat denaturation of DNA did not influence the extent of binding of either ergot alkaloid derivative used.
This interaction with either double- or single-stranded DNA was decreased at high ionic strength, low pH or in the presence of 8 M urea, suggesting that electrostatic interactions and hydrogen bonds may be involved in the formation of the complex.

Introduction

Many of the small molecules such as enzyme substrates and inhibitors, as well as numerous physiologically and pharmacologically active substances may interact in different ways with macromolecular cell components. These interactions are of fundamental importance in biology, because biological effects of some of these molecules can be correlated with their binding to macromolecules, especially to nucleic acids.

Ergot alkaloids and many related compounds have been used for decades as pharmacologically active agents. Recently published data /9/ concerning pharmacological effects of EmS (Fig. 1, graph A) and EnmS (Fig. 1, graph B) suggest the possibility of their usage in migraine treatment.

A number of authors /5, 8, 11/ have reported that a related compound, lysergic acid diethylamide (LSD), causes chromosomal

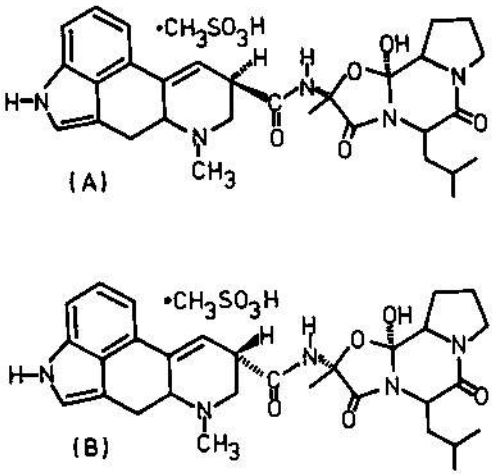

FIG. 1 Structure of EmS (graph A), and EnmS (graph B)

breaks both in vivo and in vitro. Although the importance of these findings needs further experimental evidence, the results suggest the possibility that this substance may interact with some chromosomal components. SMYTIES and ANTUN /16/, WAGNER /18/, and YIELDING and STERNGLANZ /19/ found connections between the interaction of this agent with DNA and birth deffects.

The investigations described in this paper have been performed with the aim to shed more light on the mechanism of action of EmS and EnmS at the molecular level. Accordingly, purified DNA, poly(U), poly(A), sRNA and GMP have been examined for their ability to bind these ergot alkaloid derivatives. On the basis of these results obtained by in vitro studies of interaction of these compounds with the native calf thymus DNA, one could speculate about the possibilities of side effects in the therapeutical application of both lysergic and iso-lysergic acid derivatives.

Material and Methods

EmS and EnmS were a gift of "LEK" Pharmaceutical and Chemical Industry, Lyublyana Yugoslavia. Concentration of stock solutions of both EmS and EnmS, prepared in 0.01 M Na-phosphate buffer, pH 6.1, were 0.5×10^{-3} M. They were determined on the basis of the molar absorption in methanol at 312 nm, according to SMITH and TIMMIS /14/ and STALL et al. /17/.

Sodium salt of native calf thymus DNA (a "SIGMA" product) was dissolved in 0.01 M Na-phosphate buffer, pH 6.1, to a concentration of 1.7×10^{-3} M. To get a homogeneous solution (to enable the use of high DNA concentrations without excessive viscosity /12/) it was three times passed through the G-25 injection needle. Different volumes of DNA solution were added to either EmS or EnmS,

the final concentration of either ergot alkaloid derivatives being 0.5×10^{-4} M, total volume of experimental mixture 10 ml. Absorption spectra were recorded from 290-360 nm at 25 °C, against corresponding DNA solution.

Spectrophotometric titrations of both ergot alkaloid derivatives with DNA were performed at 312 nm, using a combination of Beckman DU monochromator and Gilford detection system. EmS or EnmS 0.5×10^{-4} M solutions were titrated with the graded DNA solutions from 1.7×10^{-4} - 34.6×10^{-4} M. The data were further processed as suggested by BLAKE and PEACOCKE /3/. If the absorption of the free alkaloid (total concentration c_t) is D_t, absorption of totally bound alkaloid is D_b. For a system with clear isosbestic point, coefficient α_i is:

$$\alpha_i = \frac{D_t - D_i}{D_t - D_b}$$

were D_i represents absorption of alkaloid in the mixture. Concentration of free, unbound alkaloid (c_{fi}) and mols of bound alkaloid per mol of nucleotide (r_i) are: $c_{fi} = (1 - \alpha_i) \cdot c_t$ and $r_i = \frac{\alpha_i \cdot c_t}{c_n}$, where c_n is concentration of nucleotides.

Binding parameters were obtained by the graphic method of SCATCHARD /13/. In this way it was possible to calculate intrinsic association constants, as well as the number of binding sites.

Thermal stability of DNA before and after ergot alkaloid derivatives addition was determined measuring absorption changes at 260 nm. Samples were heated in thermocuvettes connected to a thermoprogrammer, Gilford model 2527, at a constant rate of 1 °C per minute. Reference solutions contained corresponding concentration of ergot alkaloid derivative. Changes of ergot alkaloid derivative absorption were subtracted from these of DNA-alkaloid derivative mixture.

Examination of interaction of both EmS and EnmS with denatured DNA, as well as the influence of ionic strength of the medium on this process, were performed by determination of binding coefficient f_b, defined by ARYA and YANG /1/ as: $f_b = \frac{c_b}{c_t \cdot c_n}$, or according to BLAKE and PEACOCKE /3/: $f_b = \frac{\alpha_i}{c_n}$, where f_b represents a fraction of alkaloid bound per mole of nucleotide, c_b concentrations of bound alkaloid, c_t total alkaloid concentration, and c_n concentration of nucleotides. These data were obtained by spectrophotometric titration at 312 nm. Denatured DNA was prepared heating DNA solution for 10 min at 100 °C and rapid cooling.

Results

Absorption spectra of pure EmS and EnmS dissolved in 0.01 M Na-phosphate buffer, pH 6.1, together with the spectra of their mixtures with DNA and the spectra of completely bound ligand to DNA are presented in Fig. 2. From this figure it can be seen that the solutions of pure ergot alkaloid derivatives display one absorption maximum at 312 nm, which is decreased after addition of DNA. In the case of completely bound alkaloid, resolution of this maximum occurs, and two maxima at 293 and 320 nm appear. Clear isosbestic points for the mixtures studied were at 350 and 345 nm for EmS and EnmS, respectively.

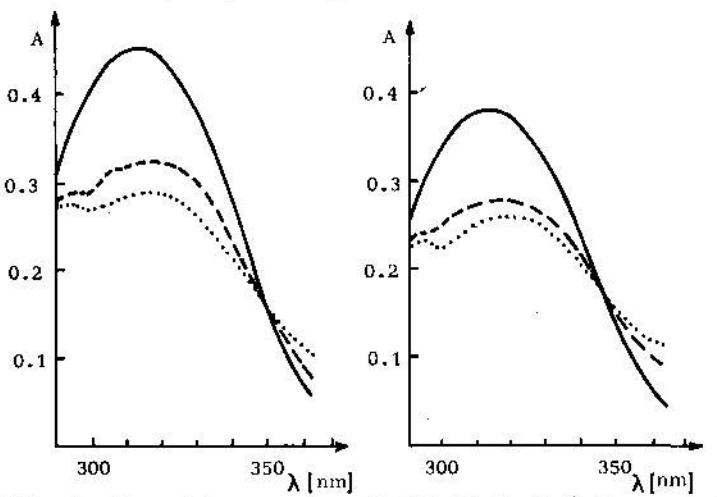

FIG. 2 Absorption spectra of EmS (0.49×10^{-4} M, graph A) and EnmS (0.44×10^{-4} M, graph B) before (solid line) and after (dashed line) addition of DNA (3.36×10^{-4} M), or after addition of 35.6×10^{-4} M of DNA (dotted line)

Spectrophotometric titrations of both EmS and EnmS were performed at 312 nm, because this wavelength corresponds to one of the characteristic absorption maxima of ergot alkaloids and their derivatives. DNA absorption at 312 nm, although low, was always subtracted from the absorption of the mixture. The data obtained for the light absorption of both EmS and EnmS, in a defined concentration, light absorption of totally bound alkaloid and that of the mixtures of these compounds and DNA were used for SCATCHARD /13/ plot analysis of their interaction with DNA (Table 1). These results are presented in Fig. 3.

TAB. 1 Binding parameters of EmS and EnmS interaction with the native calf thymus DNA

Binding parameter	EmS	EnmS
Intrinsic association constant, K_a	$(1.6\pm0.1)\times10^5\ M^{-1}$	$(1.4\pm0.1)\times10^5\ M^{-1}$
No. of binding sites per mole of nucleotides, n	0.04	0.04
Standard free energy change $(-\Delta G_o)$	$30.14\ kJM^{-1}$	$29.72\ kJM^{-1}$

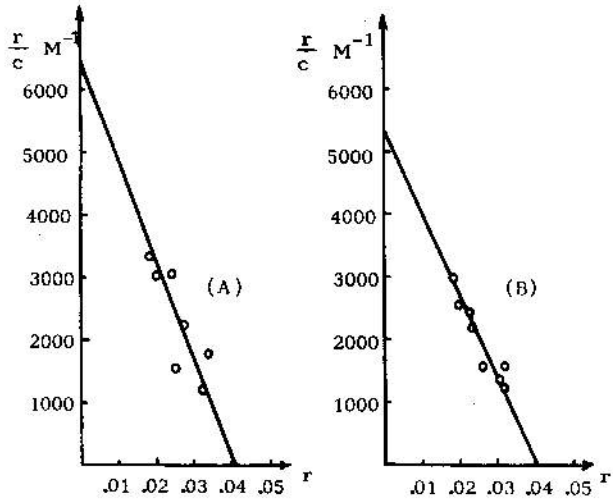

FIG. 3 SCATCHARD /13/ plot analysis of interaction of EmS (graph A) and EnmS (graph B) with the native calf thymus DNA. r = moles of alkaloid derivative bound per mol of nucleotides. c = concentration of unbound alkaloid.

From the data presented in Fig. 3 and Tab. 1, it can be concluded that both EmS and EnmS interact with the native calf thymus DNA in a similar manner.

Fig. 4 represents derivative melting curves of DNA before and after addition of EmS (graph A) and EnmS (graph B). Mild stabilisation of DNA double helical structure in the presence of either substance used can be seen. The degree of this stabilisation was proportional to the amount of bound ergot alkaloid derivative. The differences of DNA melting points (T_m) resulting from its inter-

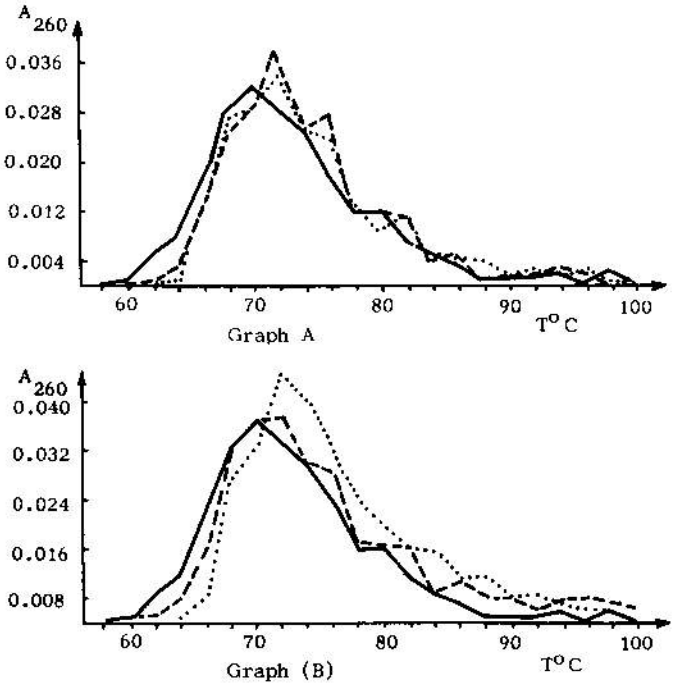

FIG. 4 Derivative thermal denaturation curves of DNA before and after addition of ergot alkaloid derivatives.
Graph A - Solid line - 0.86×10^{-4} M DNA; dashed line - after addition of EmS (final concentration 0.5×10^{-4} M); dotted line - after addition of the same compound to a final concentration of 1.1×10^{-4} M.
Graph B - The details are the same as given in caption of graph A, only EnmS was used instead of EmS.

TAB. 2 Effect of binding of EmS and EnmS to DNA on its thermal stability

Ratio EmS/DNA	T_m increase	Ratio EnmS/DNA	T_m increase
0.58	0.9°C	0.58	0.5°C
1.28	1.1°C	1.28	2.2°C

action with ergot alkaloid derivatives are given in Table 2. The effects of ionic strength (μ) of the medium, of polynucleotide structure, and presence of denaturing agents, such as 8 M urea, and very low pH, on the interaction of the compounds examined are

presented in Table 3. It can be seen that the increase of μ significantly decreases binding coefficients of both EmS and EnmS. However, denaturation of DNA did not affect this process, while 8 M urea, or low pH values, influenced partially decreased interaction of both ergot alkaloid derivatives with DNA as can be seen from the binding coefficients given in Table 3.

TAB. 3 Effect of ionic strength and denaturation of DNA on its interaction with ergot alkaloid derivatives

Polynucleotide	μ	pH	$f_b \times 100\ M^{-1}$ (EmS)*	$f_b \times 100\ M^{-1}$ (EnmS)*
Double-stranded DNA	0.02	6.1	12	12
Double stranded DNA	0.24	6.1	2	2
Double stranded DNA in 8 M urea	0.02	6.1	4	5
Heat denatured DNA	0.02	6.1	14	13
DNA denatured lowering pH	0.02	2.4	7	8

*Binding coefficients (f_b) were calculated according to ARYA and YANG /1/. Experimental error $\pm 1 \times 10^2\ M^{-1}$.

The binding of EmS and EnmS to poly(U), poly(A), yeast sRNA and GMP was very low and in the range of experimental error. This was estimated by considering their hypochromic effects. These effects amount to about 0.5%, 1%, 5% and 0% for poly(A), poly(U), yeast sRNA and GMP, respectively, while for DNA or denatured DNA to 33%.

Discussion

Interaction of biologically active molecules with nucleic acids has been usually investigated in order to get a better insight into the conformation which nucleic acids are capable of adopting, or to contribute to our knowledge of the mechanism of action of bioactive molecules. Several classes of different compounds such as dyes /10/, antibiotics /12, 15/, carcinogenic hydrocarbons /2, 4, 7/, alkaloids /6, 20/, *etc.* bind to nucleic acids. They often show the preference for particular conformation and base specificity. However, only in a limited number of instances has the

interaction of alkaloids and their derivatives with DNA been investigated. This prompted us to study the formation of a complex with DNA of two synthetic ergot alkaloids, EmS and EnmS, with presumed antimigrainic activity /9/.

The results obtained show that native calf thymus DNA alters the absorption spectra of both alkaloid derivatives used and intensity of absorption bands was decreased after addition of DNA. These results could be connected to earlier findings of other authors who have studied the interaction of double-stranded DNA with actinomycin /12, 15/ and chloroquinone /6, 20/, as well as to analogous intercalating of LSD with DNA /19/.

Different steric structure and the presence of the peptide part of the molecule in comparison with the simpler lysergic acid derivatives, led to different interaction of both EmS and EnmS with DNA. Receptor sites for these compounds are much more specific than in the case of diethylamide of lysergic acid (binding ratio for the latter being 1 : 1 /19/). Different mode of interaction of ergot alkaloid derivatives and DNA (double- or single-stranded) in comparison with that of LSD or 2-Br-LSD might be the result of the presence of carboxamide (peptidic -NH) groups in ergot alkaloids.

The results obtained at high ionic strength, or in the presence of 8 M urea, as well as at low pH, support the idea that alkaloid derivatives studied request the oxygen of deoxyribose ring to interact with their peptidic -NH groups, forming hydrogen bonds, similar to interaction of cyclic peptides with DNA /12/. Low extent of interaction of both EmS and EnmS with poly(A), poly(U), sRNA and GMP indicate that ribonucleosides are not capable of forming the complex with either ergot alkaloid derivative used.

If we presume, in accordance with already known data for LSD and LSD-25 /2/, that an intercalation model is valid (which is neither confirmed nor rejected by our results), the lower number of binding sites for EmS and EnmS on DNA molecule than for LSD could be related to the steric hindrance of the bulky side group of the formers. The pronounced influence of increasing ionic strength on this process points to the same direction.

Hypochromic and bathochromic effect of DNA on ergot alkaloid derivatives absorption could result from stacking of alkaloid molecules between DNA bases in a polymeric array /21/. However, it should be taken into account that solely spectroscopic arguments

cannot lead to definite conclusions concerning the type of interaction, because absorption spectra merely reflect the electronic environment of the molecule and do not give specific information about the type of interaction.

Binding coefficients obtained in experiments with double- and single-stranded DNA were about the same, meaning that, for interaction of EmS and EnmS with DNA, double helical structure of DNA is not necessary.

Our results demonstrate that interaction of ergot alkaloid derivatives studied occurs even at very low concentrations of these substances. This can be attributed to the presence of the receptor site on DNA molecule, which enables π-electron layer interaction as well as the formation of hydrogen bonds. The analysis of this interaction under in vivo conditions will be along the line of our further interest.

Acknowledgement

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In vitro изучалась интеракция ергозин метансульфоната (ЕмС) и ергозинин метансульфоната (ЕнмС) с двухспиральной и денатурированной ДНК.

Изменение в апсорпционных спектрах двух алкалоидных деривато в при получений ДНК, потом результаты спектрофотометрических титрациях этих веществ с ДНК как и повышение термической стабильности ДНК в наличий этих алкалоидных веществ, дают тачно определённые реакций. Внутерннье константы асоциаций $1,6x10^{5}M^{-1}$ и $1,4x10^{5}M^{-1}$. Номер связывающих мест 100 молов нуклеотида состав-4 для ЕмС и для ЕнмС. Тепловая денатурация ДНК не оказивает влияния на обьем связывания алкалоидних веществ.

Интеракция с одной или двухспиральной ДНК уменшается при высокой йоной силе, маленьким pH и в присуствии 8M урее. Это означает, что оформление комплекса - причина електростатических интеракций и водородных связей.

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