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INTERACTION OF SEVERAL ERGOPEPTINES WITH CALF THYMUS DNA IN VITRO

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Interaction of ergosine (ESN), ergosinine (ESNN) and dihydroergosine (DHESN) methanesulphonates with native and single-stranded DNA was studied by equilibrium dialysis method at 8°C. It was found that the interaction of these ergot alkaloids with native DNA is a rather complex process for ESN and ESNN. Two different types of binding sites on DNA, with intrinsic association constants of the same order of magnitude, but with very different Hill coefficients for the cooperativity were identified. Parameters for binding of alkaloids to native DNA were K_1=17950 M^{-1}, n_1=0.050, n_{H1}=0.67, K_2=17530 M^{-1}, n_2=0.035, n_{H2}=12.0 for ESN, K_1=11000 M^{-1}, n_1=0.066, n_{H1}=1.00, K_2=15000 M^{-1}, n_2=0.022, n_{H2}=15.0 for ESNN. Binding parameters for interaction of native DNA with DHESN were: K=8870 M^{-1}, n=0.150, n_H=1.11.

The extent of binding of ergopeptines was higher when single stranded DNA was used in comparison with that obtained with the native DNA. High ionic strength of the medium, diminished interaction of ergopeptines with DNA, similar to the data obtained in the presence of 8M urea or when binding of ergot alkaloids with wheat germ mRNA was examined. Derivative melting curves of calf thymus DNA before and after addition of DHESN demonstrated change in stabilisation of double helical DNA structure which was proportional to DHESN concentration.

INTRODUCTION

Numerous biologically and pharmacologically active compounds interact with double-stranded DNA with different affinities, different specificities and in a variety of ways /22/ and it was suggested that biological activity is directly dependent on both specific affinities and the particular mode of binding /8/. In connection with the aforementioned facts, it is obviously very important to define and characterize the nature of drug-DNA interactions, not only in order to understand better molecular mechanisms underlying their pharmacological activities, but also to check whether such interactions lead to the damage of genetic material.

It is well documented that lysergic acid diethylamide (LSD) causes chromosomal breaks both *in vivo* and *in vitro* /4,10,16/ and several authors /21,23/ demonstrated connections between the interaction of this agent with DNA and birth defects.

In our previous work /11/, we observed specific interactions of two synthetic ergo-

peptines, ergosine (ESN, derivative of lysergic acid) and ergosinine (ESNN, derivative of iso-lysegic acid) and denatured DNA under in vitro conditions by spectrophotometric analyses. In this paper the modes of binding of ESN, ESNN and dihydroergosine (DHESN, derivative of dihydrolysergic acid) to native and denatured DNA were comparatively studied by the equilibrium dialysis method and it was shown that modifications of both steric and chemical structure of ergopeptines examined, influenced the extent of their binding.

MATERIAL AND METHODS

ESN, ESNN, and DHESN methanesulphonates were kindly donated by "Lek" Chemical and Pharmaceutical Industry, Ljubljana, Yugoslavia. They were dried over anhydrous $CaCl_2$ for 74 hrs, weighed, and dissolved in 0.01 M Na-phosphate buffer (NaPB), pH 6.1, to give $1.0\text{-}2.8\times10^{-4}$ M stock solutions. Molar absorptivities of ESN and ESNN solutions were determined at 312nm (20^{0}C, solution in 0.01 M NaPB, pH 6.1) on the basis of corresponding molar absorption in methanol at 312nm /18,20/. Molar absorptivity coefficient of DHESN was determined at 279nm (under the same conditions). All ergopeptines examined obey Beer's law within the concentration range applied.

Sodium salt of native calf thymus DNA (a "Sigma" product) was dissolved in 0.01 M NaPB, pH 6.1, to give a concentration of about $1\text{-}4\times10^{-3}$ M. Single-stranded DNA was prepared by thermal denaturation of native DNA solution (10 min, 100^{0}C) followed by rapid cooling in an ice bath, or by 8M urea, or by lowering pH of the solution.

Wheat germ mRNA solution (a "Calbiochem" product) was prepared in an identical manner with DNA solution.

Interactions of ergot alkaloids examined with the native and denatured DNA, as well as with mRNA were studied by optical and equilibrium dialysis methods. Spectrophotometric analyses were performed using a combination of Beckman DU monochromator and Gilford detection system.

Equilibrium dialysis was carried out in 0.01 M NaPB, pH 6.1, at 8^{0}C as recommended by KLOTZ et al. /13/. The data obtained for the interaction of the denatured DNA were further processed using Scatchard equation: $r/c=K(n-r)$ where r represents moles of ergot alkaloids bound per mole of nucleotides, K is intrinsic association constant, c is concentration of unbound alkaloids and n, number of binding sites (moles of alkaloid bound per mole of nucleotides at saturation).

To evaluate binding parameters for the interaction of native DNA, experimental data were fitted with the Hill equation for a system having different types of binding sites, which do not involve multiple contacts, as it was discussed by SCHWARZ /14/:

$$r(c)=n_1 \frac{(K_1c)^{nH1}}{1+(K_1c)^{nH1}} + n_2 \frac{(K_2c)^{nH2}}{1+(K_2c)^{nH2}}$$

where n_H represents Hill's coefficient of cooperativity for a system with single or two types of binding sites. K represents intrinsic association constants, and n is the number of binding sites. All these parameters were optimized using our computer program based on Davidon-Fletcher-Powell method /15/.
Thermal stability of DNA before and after addition of DHESN was determined as described previously for ESN and ESNN /11/.

RESULTS

The absorption spectrum of pure DHESN-methanesulphonate dissolved in 0.01M NaPB, pH 6.1, is presented in Fig.1.

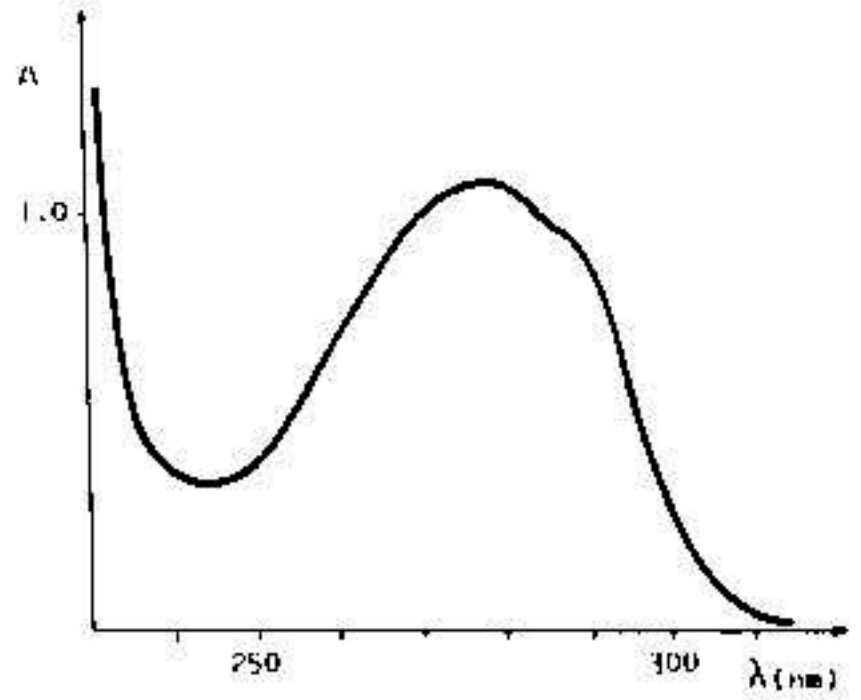

FIG. 1 Absorption spectrum of DHESN (1.8×10^{-4} M NaPB, pH 6.1)

As seen from Fig.1, DHESN displays one absorption maximum at 279 nm, where DNA solution expresses a strong absorption, as well. Due to this interference, it was not possible tc examine the interaction of DHESN with DNA by simple spectrophotometric titration. Therefore, the data obtained by equilibrium dialysis were used for direct plot analysis (r=f/c/), depicted in Fig.2.
The data presented in Fig.2 show that the extent of interaction of ergot alkaloids is higher when single-stranded DNA was used than when native DNA was applied. High ionic strength diminished the extent of DNA interaction with ergopeptines examined. Similar results were obtained in the presence of 8M urea, or when interaction with wheat germ mRNA was studied. Binding parameters obtained by curve fitting of the Hill equation are listed in Table 1. Derivative melting curves of DNA before and after addition of DHESN are presented in Fig.3. A certain effect on double-helical DNA structure after addition of DHESN can be seen.

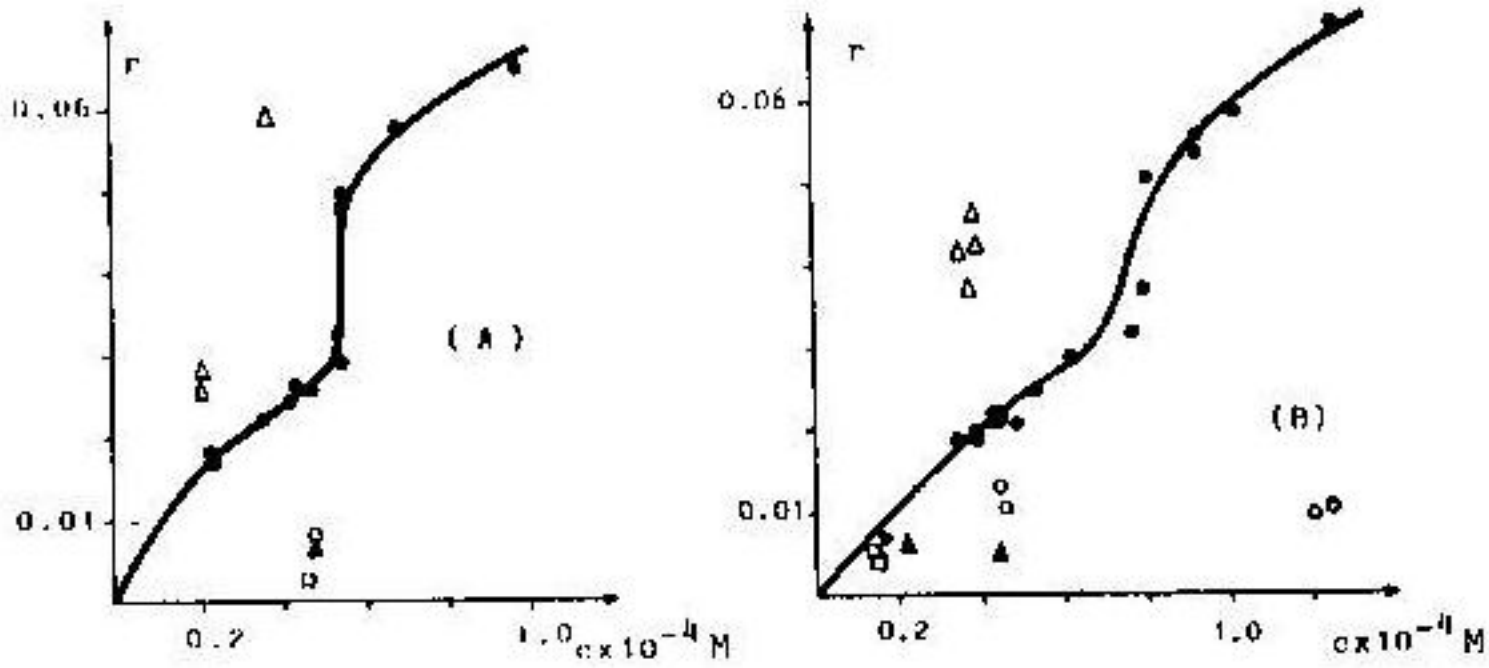

FIG. 2 Direct plot analysis for the interaction of ESN (A), ESNN (B) and DHESN (C) with the native calf thymus DNA in 0.01M NaPB, pH 6.1 (●). Points falling out of the curve represent r=f(c) values for interaction of ergopeptines examined with native DNA at high ionic strength (□), u=0.24; heat denatured DNA (△) (high values of r are not plotted); DNA in the presence of 8M urea (▲); interaction with wheat germ mRNA (○)

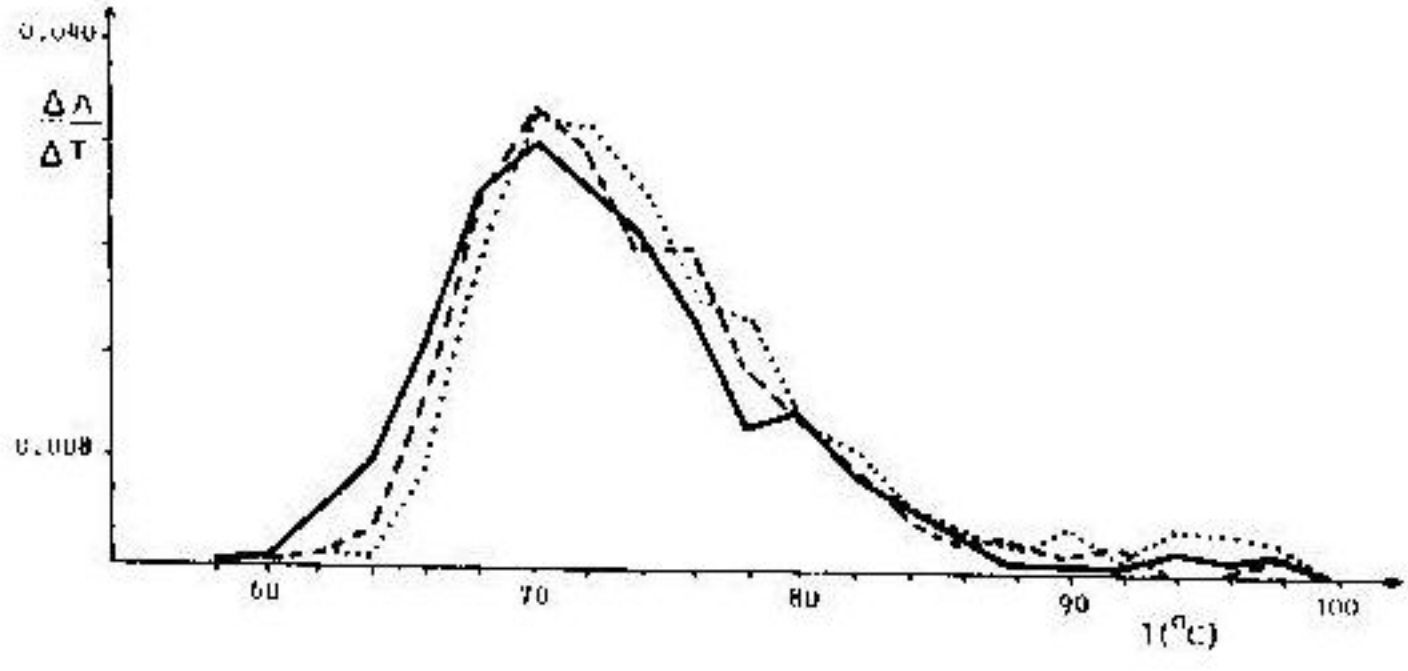

FIG. 3 Derivative melting curves of DNA before (solid line) and after addition of DHESN (dashed line) to a final concentration of 0.5×10^{-4}M, and after addition of DHESN (dotted line) to a final concentration of 1.0×10^{-4}M. DNA concentration was 0.86×10^{-4}M

TABLE 1 Binding parameters for the interaction of ESN, ESNN and DHESN with the native calf thymus DNA calculated by direct plot analysis at 8°C and equilibrium conditions

Binding parameter	Ergopeptine ESN	ESNN	DHESN
Intrinsic association constants $[M^{-1}]$			
K_1	17950±2510	11000±450	8870±615
K_2	17530±2450	15000±620	-
Number of binding sites per mole of nucleotide			
n_1	0.050±0.007	0.066±0.003	0.150±0.001
n_2	0.035±0.005	0.022±0.002	-
Hill coefficient of cooperativity			
n_{H1}	0.67	1.00	1.11
n_{H2}	12.0	15.0	-

DISCUSSION

It is well known that ergot alkaloids and their synthetic derivatives exert diverse pharmacological effects in relation to small changes of their chemical structure /5/. This group of compounds affects the central nervous system by interaction with plasma membrane receptors for a number of monoamines /1-3,6,9/. However, although LSD is known to interact with DNA /25/, and ergot alkaloids can be considered as derivatives of lysergic acid, the data concerning possible interactions of these compounds with DNA are missing in the available literature. Our previous studies /11/ demonstrated that ESN and ESNN bind to calf thymus DNA under in vitro conditions in a specific way and characteristic binding parameters were determined. In this work these examinations were extended, and besides ESN and ESNN, interaction of DHESN with double- and single-stranded DNA as well as with wheat germ mRNA was investigated, using the equilibrium dialysis method. It was found that the interaction of these ergot alkaloids with the native DNA is rather complex for ESN and ESNN and two different types of binding sites on the DNA, with the intrinsic association constants of the same order of magnitude and very different Hill's coefficients of cooperativity were identified.

Binding parameters were n_1=0.050, K_1=17950 M^{-1}, n_{H1}=0.67 for ESN and n_1=0.066, K_1=11000 M^{-1}, n_{H1}=1.00 for ESNN indicating the reactive sites with mild negative or no cooperativity, and n_2=0.035, K_2=17530 M^{-1}, n_{H2}=12.0 for ESN and n_2=0.022, K_2=15000 M^{-1}, n_{H2}=15.0 for ESNN indicating the other group of binding sites with

very high cooperativity. Binding parameters for the interaction of DHESN with native DNA were n=0.150, K=8870 M^{-1}, n_H=1.11 which indicates a process with mild cooperativity.

Numbers of binding sites for ESN and for ESNN per mole of nucleotide at saturation calculated on the basis of data obtained by the equilibrium dialysis method for the process with low cooperativity were practically identical with those obtained when the spectrophotometric method was used (n=0.06 and 0.04, respectively).

Equilibrium dialysis method enabled to perform the analysis with higher alkaloid concentrations. Under these conditions it was possible to obtain higher values of parameter r for the interaction of alkaloids with DNA. In this way the existence of binding sites on DNA with high cooperativity, which were less involved in the reaction at lower alkaloid concentrations (as in spectrophotometric experiments /11/), was observed.

Denatured DNA reacts with the aforementioned alkaloids in a highly cooperative manner (Scatchard plot is represented by relation r/c=Kxr /14/). Values of intrinsic association constants calculated on the basis of Scatchard plot analysis of binding data were: $K_{(ESM)}$=45500±1100 M^{-1}, $K_{(ESNN)}$=27500±1100 M^{-1}, $K_{(DHESN)}$=15870±230 M^{-1}. Intrinsic binding constants, K, were higher for the interaction of examined alkaloids with the denatured than with the native DNA. The number of binding sites, n, for the interaction with denatured DNA was not evaluated because of limited solubility of alkaloids, so that it was not possible to approach experimentally complete saturation of denatured DNA. (Semilogarithmic analysis of binding data for the interaction with the native DNA indicates that the primary binding process of negative or very low cooperativity might create some parts of denatured regions on DNA reacting in a highly cooperative manner).

It was found that denaturation of DNA abolishes interaction with either LSD or 2-Br-LSD /25/. Our results suggest additional interaction with single-stranded DNA, which accordingly could mean that it involves hydrogen from carboxamide or some other active group(s) of the peptide part of the ergot alkaloid molecule.

Decreased extent of ergot alkaloid binding to DNA in the presence of 8M urea suggests the importance of hydrogen bond formation for this interaction. Low binding of ergopeptines examined with wheat germ mRNA (differences were within experimental error) could mean that deoxyribose or deoxyribonucleotides are involved in this interaction. High ionic strength of the medium diminished also the extent of ergot alkaloids binding to DNA very probably because of electrostatic repulsion of alkaloid cations, preventing their approach to the active sites on DNA molecule.

KIDRIČ et al. /12/ and WEBER et al. /23/ demonstrated a dependence of steric configuration on D-ring conformation. We propose that the steric conformation of ergot alkaloids influences their interaction with DNA molecules. Substituents in position C-8 are oriented pseudoequatorially and pseudoaxially for ESN and ESNN mole-

cules, respectively. Due to the interaction of carboxamide group hydrogen in position 6 of the molecule, the orientation of this substituent could be easily changed to corresponding beta- i.e. alphaaxial. In this way the peptide part gets closer to the heterocyclic moiety of the molecule and the entire molecule becomes more globularly shaped, rendering approach to individual active ergopeptine groups more difficult, which results in a relatively low number of binding sites registered throughout our work for both ESN and ESNN. Interaction of carboxamide hydrogen with N-6 would require transition of the D-ring from stable to less stable boat conformation and, connected to that, this molecule retains an elongated shape enabling all active groups to interact with DNA without more serious steric interferences. These considerations could explain the more efficient binding of DHESN to DNA in comparison with that observed for both ESN and ESNN during our studies.

Lower association constant values for ESN and ESNN interaction with the native DNA at 8°C than at 25°C observed earlier /11/ indicate the positive change of the enthalpy of the process. This finding together with a more efficient ergopeptine interaction with single-stranded DNA could be interpreted as a result of partial denaturation of the native DNA molecule during the interaction of ergot alkaloids with DNA /17/.

REFERENCES

/1/ BERDE, B., SHILD, H.O., In: "Ergot Alkaloids and Related Compounds" (1978) Springer, Heidelberg
/2/ BÜRKI, H.R., ASPER, H., RUCH, W., ZÜGER, P.E., Psychopharmacol., 57 (1978) 227
/3/ CLOSSE, A., BOLLIGER, G., DRAVID, A., FRICK, W., HAUSER, D., PFAFLI, P., SAUTERS, A., TOBLER, H.J., In: "Molecular Pharmacology of Neurotransmitter Receptors", (1938) Segawa et al. eds., Raven Press, New York
/4/ COHEN, M.M., MARINELLO, M.J., BACK, N., Science, 155 (1967) 1417
/5/ FLÜCKIGER, E., VIGOURET, J.M., WAGNER, H.R., In "Progress in Prolactin Physiology and Pathology", (1978) Robin, C., Harter, M., eds., North-Holland Biomedical Press, Amsterdam
/6/ FUXE, K., FREEDHOLM, B.B., ÖGREN, S.O., AGNATI, L.F., HÖKFELT, T., GUSTAFSSON, J.A., Fed.Proc., 37 (1978) 2181
/7/ GOLDSTEIN, M., LIEBERMAN, A., Lew, J.Y., ASANO, T., ROSENFELD, M.R., MAKMAN, M.H., Proc.Natl.Acad.Sci., U.S.A., 77 (1980) 3725
/8/ HAHN, F.E., In: "Antibiotics 3", (1975) p.79, Corcoran, J.W, and Hahn, F.E. eds., Springer Verlag, New York
/9/ HRUSKA, R.E., SILBERGELD, E.K., J.Neurosci.Res., 6 (1981) 1
/10/ IRWIN, S., EGOZCUE, J., Science, 157 (1967) 1417
/11/ JURANIĆ, Z., KIDRIČ, M., JURANIČ, I., PETROVIĆ, J., Studia Biophysica, 95 (1983) 183
/12/ KIDRIČ, J., GRAD, M., HADŽI, D., Proc. 22 Amperè Congress on Magnetic Resonance and Related Phenomena, Zürich, Switzerland 1984
/13/ KLOTZ, I.M., WALKER, F.M., PIVAN, R.B., J.Am.Chem.Soc., 68 (1946) 1486
/14/ SCHWARZ, G., Biophys.Struct.Mechanisms, 2 (1976) 1
/15/ FLETCHER, R., POWELL, M.J.D., Computer J., (1963) 163
/16/ LOUGHMAN, W.D., SARGENT, T.J., ISRAHSTAM, D.M., Science, 158 (1967) 508

/17/ McGEE, J.D., Biopolymers, 15 (1976) 1354
/18/ SMITH, S., TIMMIS, G.M., J.Chem.Soc., (1937) 396
/19/ SMITHIES, J.E., ANTUN, F., Nature, 223 (1969) 1061
/20/ STALL, A., HOFFMANN, A., PETRZILKA, T.H., Helv.Chim.Acta, 34 (1951) 1544
/21/ WAGNER, T.E., Nature, 222 (1960) 1170
/22/ WARING, M.J., Ann.Rev.Biochem., 50 (1981) 159
/23/ WEBER, H.P., LOOSLI, H.J., PETCHER, T.J., Proc.Symp.Steric Effects in Biomolecules, Eger, Hungary 1981, p.39
/24/ WHITE, F.J., APPEL, J.B., Science, 216 (1982) 535
/25/ YIELDING, K.L., STERNGLANZ, H., Proc.Soc.Exptl.Biol.(N.Y.), 128 (1968) 1096

Исследована интеракция методом равновесного диализа на 8°C эргозин - (ЭСН); эргозинин - (ЭСНН) и дихидроэргозин - (ДХЭСН) метансульфоната с нативной и одноцепочной ДНК. Показано, что интеракция является очены сложной. На ДНК определены два различных местах связывания со свойственными константами ассоциации самого порядка размера, но совсем различными коэффициентами кооперации Хилла. Параметры связывания алкалоидов с ДНК: $к_1$ = 17950 $м^{-1}$, n_1 = 0.050, n_{H1} = 0.67, $к_2$ = 17530 $м^{-1}$, n_2 = 0.035, n_{H2} = 12.0 за ЭСН; $к_1$ = 11000 $м^{-1}$, n_1 = 0.066, n_{H1} = 1.00, $к_2$ = 15000 $м^{-1}$, n_2 = 0.22, n_{H2} = 15.0 за ЭСНН. Параметри связывания ДХЭСН с нативной ДНК: к = 8870 $м^{-1}$, к = 0.150, n_H = 1.107.

В случае применения одноцепочного ДНК обьем свьэывания был большим по отношению на нативный ДНК. Высокая ионная сила уменьшает интеракцию эргопептина и ДНК. Получены похожие результаты при исследовании связывания эргот алкалоида с РНК пшеничного зародыша. Деривационные кривые плавления ДНК тимуса теленка прежде и после добавления ДНЭСН показали некоторое изменение стабилизации структуры двойного хеликса, которое оказалось пропорциональным с концентрацией ДХЭСН.

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