INVESTIGATION OF THE ACTION OF STEROIDS ON SECONDARY STRUCTURE OF DNA AND DEOXYRIBONUCLEOPROTEIN

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A study of denaturation of rat liver nucleic acids by heat in solutions of steroids (viadril, 6-methylprednisolone, and cortisol) showed that the presence of steroids in a solution of physiological ionic strength does not significantly alter the character of thermal denaturation.

It is generally agreed that hormones influence the synthesis of protein and nucleic acids [5]. Chemical interaction between molecules of DNA and doexyribonucleoprotein (DNP) and steroids can lead to subsequent structural changes which, in some cases, can be detected by a comparative structure of the denaturation of native and modified biological macromolecules [3]. Several preliminary models of the action of hormones on gene expression have been suggested [9], but the problem is not yet finally settled. If hormones act directly on chromosomes [8] this effect can be described as action at the molecular level, at which the complementary strands of DNA, twisted into a helix, separate as the result of weakening of the cross-linkages. Absorption of the biopolymers at 260 nm is a sensitive index of the degree of separation of the strands during the action of physicochemical agents.

In the present investigation the possible action of a number of steroids on the thermal denaturation of DNA and DNP solutions under physiological conditions was investigated.

EXPERIMENTAL METHOD

Samples of rat liver DNA were obtained by a modified Marmur's method [13]. The protein content, estimated by Lowry's method [11], in the samples did not exceed 1-2%. The DNA concentration was determined spectrophotometrically. The characteristic viscosity (η) was 68 dl/g, the sedimentation constant (K_S) 20S. The samples of rat liver DNP were isolated in 0.7 M NaCl solution by the method of Mirsky and Pollister [14]. The N/P ratio was 3.6-4.2, $E_{(p)260}$ =6800-7200; η=32 dl/g. Purification and chromatographic separation of the DNA samples thus obtained were carried out on a column with aminoethylcellulose. The chief "peak" was chosen and dialyzed against the solvent until the necessary DNA concentration and ionic strength of the solution were obtained. The solvent for DNA was 0.15 M NaCl plus 0.001 M EDTA (pH 6.9) and for DNP 0.7 M NaCl (pH 7.0).

Sedimentation analysis of the DNA was carried out in a Spinco-E analytical ultracentrifuge with an ultraviolet optical system, in a concentration of 17 μg/ml and at 20°C. The molecular weight (M) was calculated from the value thus obtained by the equation:

$$0.445 \log M = 1.819 + \log (S_{20,w} - 2.7) \quad [4].$$

The viscosimetric determinations were carried out in a low-gradient three-ball Ostwald viscosimeter (β_1=69.0, β_2=45.3, β_3=22.6 sec^{-1}). All the measurements were made at a temperature of 25.0± 0.1°C.

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TABLE 1. Principal characterictics of Thermal Denaturation of Rat Liver DNA and DNP

DNA sample	Melting temperature (in deg.)	Hyperchromic effect, percent	DNP sample	Melting temperature (in deg.)	Hyperchromic effect, percent
Native DNA plus viadril	83	38	Native DNP plus viadril	90	29
$1,0\times10^{-5}$ M	83,5	39	$3,1\times10^{-6}$ M	90,5	32
$1,0\times10^{-6}$ M	84,5	38			
6-methylprednisolone:			+6-methylprednisolone		
$1,0\times10^{-5}$ M	84	39			
$1,0\times10^{-6}$ M	83,5	38	$3,1\times10^{-5}$ M	91	33
Cortisol:			Cortisol		
$3,1\times10^{-5}$ M	81	39	$0,5\times10^{-5}$ M	88	32
$1,0\times10^{-6}$ M	81,5	38			
+50% ethanol			+50% ethanol		
(0,1 ml)	83	36	(0,05 ml)	87,5	29

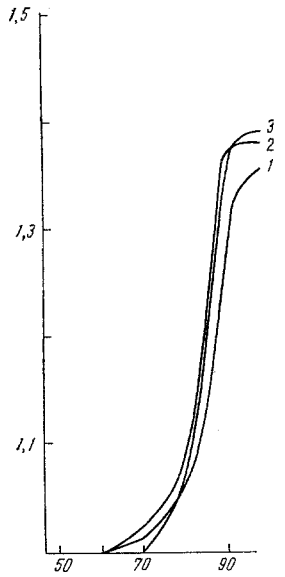

Fig. 1. Relative absorption of DNA solutions with steroids as a function of temperature: 1) DNA plus $3,1\times10^{-5}$ M 6-methylprednisolone; 2) DNA, native preparation; 3) DNA plus 1.0×10^{-5} M viadril. Abscissa, temperature (in °C); ordinate, relative absorption at λ=260 nm.

The value of M from the various preparations obtained was calculated by the equation:

$$0.665 \log M = 3.863 + \log[(\eta) + 5].$$

The choice of steroids was determined by indications in the literature of their possible action on DNA and also by their solubility in aqueous solutions. The steroids used were: 1) viadril (USA); 2) 6-methylprednisolone (West Germany); 3) cortisol (Holland). The solvents for the first two steroids were 0.15 M NaCl plus 0.001 M EDTA for reaction mixtures with DNA and 0.7 M NaCl for DNP. Cortisol was dissolved in 50% ethanol.

DNA and DNP were incubated with the steroids at 25°C for different periods of time. Thermal denaturation of DNA and DNP in the presence of steroids was carried out directly in the constant-temperature cell of a type SF-4A spectrophotometer (temperature range 20-94°C; incubation time at a given temperature 5 min).

EXPERIMENTAL RESULTS

Since DNA of animal origin is highly heterogeneous in composition, the samples of DNA isolated were chromatographically enriched on a column with aminoethylcellulose. The original DNA preparation separated on fractionation into three peaks. To assess the degree of polymerization of the purified DNA preparation (the chief "peak") sedimentation analysis was performed. The sedimentation coefficient was 20 S, corresponding to a molecular weight of 8×10^6.

It is clear from the results given in Table 1 and Fig. 1 that the steroids altered the melting temperature of both DNA and DNP. Viadril and 6-methylprednisolone, for instance, raised the melting temperature of both DNA and DNP, and the greatest stabilizing action was shown by 6-methylprednisolone in low concentrations (an increase of 3.5°C in the melting temperature compared with the original DNA preparation). By contrast with viadril and 6-methylprednisolone, cortisol had a destabilizing action, reducing the melting temperature of DNA and DNP by 3°C. Preliminary incubation of the DNA and DNP samples in the presence of steroids for different periods of time (1, 4, and 24 h) caused no subsequent changes in the character of denaturation of the DNA and DNP preparations which differed from those described already.

The melting temperature, as a function of the nucleotide composition of nucleic acids and also of the properties of the solvent (pH, ionic strength of the solution, and so on), can be used as a measure of the thermodynamic stability of the "melting" structure under the particular conditions concerned [2].

It was concluded from a comparison of the melting curves of DNA and DNP samples treated with the above-mentioned steroids under different conditions (Table 1) that the secondary structure of the DNA was altered, but not to the same extent as by the action of some antibiotics.

Results obtained by other workers [6, 10, 12] studying interaction between steroids and DNA do not contradict those of the present investigation. Differences in the character of the action of steroids on the stability of nucleic acids were evidently due to differences in their structure. To determine unequivocally whether steroids can form complexes with nucleic acids or nucleoproteins and to examine the conditions of their formation, results obtained by other methods are required. The specificity of the nucleic acids, their molecular weight, and their guanosine and cytosine composition are probably important factors [7].

The control of gene activity by the action of steroid hormones in vivo evidently incorporates more delicate regulatory mechanisms. In this respect an important role is evidently played, first, by transport proteins participating in hormonal cytoreception, and second, by repressor proteins regulating the functional state of the genes. The possibility cannot be ruled out that changes in membrane permeability for certain ions produced by hormones may activate the gene [1].

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