INTERACTION OF 17β-ESTRADIOL WITH HISTONES.

ACTION OF THE HORMONE ON PARTIALLY RECONSTRUCTED NUCLEOHISTONES

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Binding of 17β-estradiol with calf thymus histones and the effect of the hormone on thermal denaturation of partially reconstructed nucleohistones were studied. By the degree of binding of the hormone histone fractions can be arranged in the following order: $F_{2a} > F_3 > F_1 \geq F_{2b}$. Addition of 17β-estradiol caused an increase in the first melting phase and a corresponding decrease in the second melting phase of the partially reconstructed nucleohistones F_{2a}, F_3, and F_{2b}. Addition of the hormone to partially reconstructed nucleohistone F_1 caused opposite changes in the thermal denaturation profile.

KEY WORDS: 17β-estradiol; binding with histones; partially reconstructed nucleohistones; thermal denaturation.

17β-Estradiol is known to increase RNA and protein synthesis in target organs *in vivo* [1-3]. This suggests that it acts at the template level. The possibility of extraction of 17β-estradiol from crude and purified preparations of chromatin from the uterus of estrogen-treated rats [4, 5] confirms the fact that the hormone is incorporated into chromatin.

The object of this investigation was to determine the acceptor properties of chromatin proteins toward 17β-estradiol and to evaluate the role of the hormone in the change in properties of the nucleohistones.

EXPERIMENTAL METHOD

17β-Estradiol-6,7-^{3}H (Radiochemical Center, Amersham, England) and 17β-estradiol (Calbiochem, USA) were used.

Isolation and fractionation of histones from calf thymus and pig endometrium were carried out by Johns' method [6] and the purity of the histone fractions was determined electrophoretically in 15% polyacrylamide gel, pH 4.3 [7].

To determine the binding parameters of the system consisting of protein and 17β-estradiol, the protein (concentration 1 mg/ml) was incubated for 15 min with increasing doses of 17β-estradiol-6,7-^{3}H (from 10^{-14} to 10^{-11} M) in 0.002 M NaCl, pH 6.8. Free hormone was separated from bound by gel filtration on Sephadex G-25 (coarse). The quantity of hormones of the bound fraction was determined from the level of radioactivity, and the protein concentration by Lowry's method [8]. The binding constants were calculated by Scatchard's graphic method [9].

To investigate the properties of the nucleohistones, type 1 (Sigma, USA) calf thymus DNA was used after preliminary fractionation on an AE-cellulose column and elution from the resin with a stepwise concentration gradient of $MgCl_2$ (0.16, 0.32, and 0.48 M). The DNA peak eluted by 0.32 M $MgCl_2$ was used in the experiments after dialysis against the working buffer.

Reconstruction of the nucleohistones was carried out in citrate buffer (0.2 mM Na citrate, 2.8 mM NaCl, 0.05 mM EDTA), pH 6.6, with thorough mixing of the protein fractions with DNA (protein–DNA ratio for all nucleohistones 0.3). The final DNA concentration in the solution

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TABLE 1. Values of the Binding Constants of 17β-Estradiol with Histone Proteins ($K_{bind} \cdot 10^9$ M^{-1})

Source from which isolated	Temperature	Histone fraction			
		F_1	F_{2a}	F_{2b}	F_3
Pig endometrium	37°	0,35	1,02	0,31	0,75
Ditto	0°	0,17	0,31	0,23	0,32
Calf thymus	37°	0,34	0,87	0,47	0,67
Ditto	0°	0,25	0,25	0,21	0,30

was 30 μg/ml. The prepared solutions were kept at 4°C for 2 to 16 h, the air dissolved in them being removed *in vacuo*. The diffusion of light (A_{320}) did not exceed 3% of the optical density (D) of the solutions at 260 nm.

The reconstructed nucleohistones were melted in a 1-cm quartz cuvette, by heating the solution from 30 to 90°C and recording the change in D at 260 nm with a Hitachi-323 spectrophotometer every 2° after preliminary exposure for 3 min to the given temperature. The melting point of the nucleohistones (T_m) was defined as the temperature corresponding to 0.5 of the hyperchromic effect obtained during their denaturation. To determine the transition zone, a derivative of the melting curve was drawn. The temperature derivative of D was found by the equation

$$\frac{dD}{dT} = \frac{D^{260}_{T+1} - D^{260}_{T-1}}{2}.$$

The transition zone between the melting phases corresponded to the temperature at which dD/dT was minimal.

EXPERIMENTAL RESULTS

Calculations of the binding constant (K_{bind}) of 17β-estradiol with the different histone fractions, as given in Table 1, show that 17β-estradiol has high affinity for these proteins. Depending on the degree of binding of the hormone, the histone fractions can be arranged in the following order: $F_{2a} > F_3 > F_1 \geq F_{2b}$. The binding characteristics of calf thymus histones and pig endometrial histones did not differ significantly. With a change in temperature to 0°C the values of K_{bind} decreased both for endometrial histones and for thymus histones. This decrease in K_{bind} is not evidence of the enzymic nature of the binding, for the experimental conditions ensured absence of enzymes in the system. The change in the values of K_{bind} can be explained either by a decrease in the rate of complex formation or, if the incubation time was long enough to reach equilibrium, by a change in the conformational properties of the proteins and, in particular, in their hydrophobic character, which determines interaction between histones and the steroid.

The values of K_{bind} of 17β-estradiol with chromatin proteins in a free state could differ significantly from the true values of K_{bind} of the hormone with proteins incorporated into chromatin, for during binding with DNA the conformational characteristics of the histones were changed. The absence of difference in the degree of binding of 17β-estradiol with histones of the endometrium and thymus confirmed the tissue nonspecificity of these proteins.

Addition of histones to DNA in solution leads to the formation of DNP complexes, which are more resistant to thermal denaturation, as shown by the appearance of a second phase on the melting curve, the melting point of which (T_{mII}) was considerably higher than that of the free DNA. Addition of 17β-estradiol to the partially reconstructed F_1, F_{2a}, F_{2b}, and F_3 nucleohistones caused practically no change in the corresponding melting points. However, the presence of the hormone altered the distribution of the total hyperchromic effect among the melting phases of all the partially reconstructed nucleohistones. During melting of partial nucleohistone F_{2a} treated with estradiol in a concentration of 10^{-5} M, and increase in the hyperchromic effect of the first melting phase and a decrease in the hyperchromic effect of the second phase were observed. During melting of the partial nucleohistones F_{2b} and F_3, the redistribution of the hyperchromic effect caused by the hormone was similar in character but was less marked (Table 2). During thermodenaturation of the partial nucleohistone F_1, reconstructed in the presence of 10^{-5} M estradiol, on the other hand, there was an increase in the hyperchromic effect of the second melting phase and a decrease in the hyperchromic effect of the first phase.

TABLE 2. Melting Point (in deg) and Hyperchromic Effect of Melting (in %) of Partially Reconstructed Nucleohistones

Samples	Without 17β-estradiol					In the presence of 17β-estradiol					P
	T_{m_I}	$T_{m_{II}}$	H_0	H_2	H_2/H_0	T_{m_I}	$T_{m_{II}}$	H_0	H_2	H_2/H_0	
DNA	55,2±0,6	—	38	—	—	55,6±0,5	—	38	—		—
DNA + F_1	56,8±0,2	77,0±0,3	37	11	0,30	56,3±0,6	76,0±0,3	39	16	0,41	<0,05
DNA + F_{2a}	55,3±0,8	81,8±0,8	37	8	0,21	56,4±0,6	80,9±0,6	37	6	0,15	<0,05
DNA + F_{2b}	56,3±0,9	82,0±1,0	38	8	0,21	55,5±1,0	80,2±0,2	38	7	0,17	<0,02
DNA + F_3	56,0±0,5	80,5±0,6	38	8	0,20	56,2±0,2	80,2±0,1	38	7	0,17	<0,05

Legend. T_{mI} and T_{mII} represent melting points in first and second phases, respectively. H_0) Total hyperchromic effect, H_2) hyperchromic effect of second phase.

It can be postulated that the changes in the denaturation profile of the partially reconstructed nucleohistones F_{2a}, F_{2b}, and F_3 described above, caused by the addition of 17β-estradiol, were due to redistribution of the histone molecules along the DNA helix, as the result of which the DNP regions become more compact and the total length of the free DNA segments was increased. Possibly the hormone, by occupying the hydrophobic region on the histone molecule, changes its conformation so that the charged zone of the protein corresponding to this region could not enter into electrostatic interaction with the phosphate groups of DNA. The steroid could have a different action on conformational changes in histone F_1. Since the molecule of that protein is rod-shaped [10], molecules of hormones interacting with histone F_1 straighten it even more, with the result that the protein can interact with a larger region of DNA.

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