ACTION OF ESTROGENS ON RECONSTITUTED NUCLEOHISTONES

S. N. Portugalov

UDC 612.398.145.1-06:612.621.31

The action of estrone, 17β-estradiol, and estriol on the degree of binding of histones with DNA in reconstituted nucleohistones and the effect of these hormones on thermal denaturation of nucleoproteins were studied. Estrogens in concentrations of 10^{-5} M were shown not to change the protein/DNA ratio by weight in partially reconstituted F_1, F_{2a}, F_{2b}, and F_3 nucleohistones and in total nucleohistone reconstituted from DNA and an equimolar complex of histones F_{2a}, F_{2b}, and F_3. Treatment with estrogens in the above-mentioned concentration does not change the melting point of any of the nucleohistones studied. The effect of estrogens on thermal denaturation of total nucleohistone and of partially reconstituted nucleohistones F_{2a}, F_{2b}, and F_3 consists of an increase in the hyperchromic effect of the first phase of melting and a corresponding decrease in the hyperchromic effect of the other phases. Estrogens did not change the thermal denaturation profile of partially reconstituted nucleohistone F_1.

KEY WORDS: estrogens; reconstituted nucleohistones; thermal denaturation.

After administration in vivo estrogens increase the amount of mRNA available for transcription from the DNA template and consequently increase protein biosynthesis in target cells, thereby causing growth of the uterus [1, 3]. The fact that 17β-estradiol can be extracted from crude and purified preparations of chromatin from the uterus of estrogen-induced rats is evidence that the hormone is incorporated into the composition of the chromatin [8]. Although estrogens have very low affinity and low specificity for DNA [15], they have been shown to form a stable complex in vitro with the basic proteins of chromatin [2]. Comparison of all the data suggests that estrogens may act on the structural and functional organization of nucleoproteins.

The object of this investigation was to study the action of estrogens on the degree of binding of histones with DNA and to analyze the effect of these hormones on thermal denaturation of the reconstituted nucleohistones.

EXPERIMENTAL METHOD

The 17β-estradiol, estrone, and estriol used in the experiments were from Calbiochem (USA), and the calf thymus DNA was type I from Sigma (USA).

Calf thymus histones were isolated and fractionated by the method of Johns [5]; the purity of the histone fractions was verified by electrophoresis in polyacrylamide gel, pH 4.3 [9].

Nucleohistones were reconstituted by stepwise dialysis in an NaCl concentration gradient (from 2 to 0.15 M; all solutions contained Tris-HCl, pH 7.0). Individual histone fractions dissolved in 2 M NaCl or an equimolar mixture of histones F_{2a}, F_{2b}, and F_3, made up in the same solvent, were added in the required proportions by weight to a solution of DNA in 2 M NaCl. The original concentration of the DNA solution was 180 μg/ml and the protein concentration did not exceed 800 μg/ml. The original ratio of histone/DNA by weight was 1.5 for all the reconstituted nucleohistones except partial nucleohistone F_1, for which this ratio was 1. The salt concentration in the reconstitution medium was reduced successively by a series of dialyses for 3 h at 4°C against 1 liter of the corresponding solvent. The protein/DNA ratio was estimated in the resulting preparation of reconstituted nucleohistone by determining the protein concentration by Lowry's method [7] and the DNA concentration by the optical density (D) at 260 nm. Protein not bound with DNA in the course of re-

Department of Molecular Pharmacology and Radiobiology, Medico-Biological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 4, pp. 416-419, April, 1978. Original article submitted April 12, 1977.

 0007-4888/78/8504-0450$07.50

TABLE 1. Action of Estrogens on Degree of Binding of Histones with DNA in Reconstituted Nucleohistones (M ± m; n = 4-6)

Sample	Protein/DNA ratio if reconstituted Nucleohistone			
	without hormone	in presence of 10^{-5} M estrogen		
		estrone	estradiol	estriol
DNA+($F_{2a, 2b, 3}$)	1,09±0,015	1,08±0,005	1,06±0,03	1,07±0,01
DNA+F_1	0,73±0,03	0,72±0,03	0,69±0,02	0,71±0,00
DNA+F_{2a}	1,08±0,04	1,09±0,04	1,05±0,05	1,06±0,03
DNA+F_{2b}	1,12±0,02	1,13±0,01	1,13±0,03	1,10±0,02
DNA+F_3	1,06±0,03	1,07±0,03	1,04±0,05	1,08±0,04

Legend. P > 0.05.

TABLE 2. Distribution of Total Hyperchromic Effect among Phases of Melting of Reconstituted Nucleohistones (M ± m; n = 4-6)

Sample	Control		After treatment with estrogens					
			estrone		estradiol		estriol	
	H_0	H_1/H_0	H_0	H_1/H_0	H_0	H_1/H_0	H_0	H_1/H_0
DNA	37,8±0,8	1,0	37,4±0,7	1,0	36,7±1,1	1,0	38,4±1,1	1,0
DNA+F_1	36,8±0,6	0	38,6±0,5	0	39,2±1,2	0	37,4±0,9	0
DNA+F_2	38,2±1,4	0,42±0,02	37,4±1,1	0,52±0,01	37,2±1,0	0,55±0,02	37,8±0,6	0,49±0,02
DNA+F_{2b}	38,6±0,9	0,37±0,02	39,8±0,6	0,44±0,01	39,0±1,4	0,47±0,01	39,4±0,6	0,41±0
DNA+F_3	37,2±0,6	0,35±0,01	37,2±0,7	0,43±0,02	38,6±0,6	0,46±0,01	36,8±0,6	0,40±0,01
DNA+($F_{2a, 2b, 3}$)	39,3±0,4	0,39±0,01	37,8±0,3	0,47±0,02	37,3±0,9	0,4[illegible]±0,01	37,0±0	0,44±0

Legend. 1. H_0) Total hyperchromic effect of melting (in %); H_1/H_0) hyperchromic effect of first phase of melting as a fraction of total hyperchromic effect. 2. P ≤ 0.05.

constitution was separated from the nucleoprotein by gel-filtration on Sephadex G-75. The true histone/DNA ratio in the preparation was calculated from the DNA and protein content in the sample corresponding to the maximum on the nucleohistone elution profile.

To study the action of estrogens on the degree of binding of histones with DNA in the reconstituted nucleohistones an alcoholic solution of estrone, estriol, or 17β-estradiol, in a concentration of 10^{-5} M, was added to 1 ml of nucleohistone solution. The alcohol concentration in the samples dod not exceed 5%. The resulting mixture was stirred and kept at 4°C for 12 h. The nucleohistone treated with the hormone was then fractionated on Sephadex G-75 as described above.

Intact and estrogen-treated preparations of nucleohistones, characterized by reference to their stoichiometric protein/DNA ratio in the course of fractionation, were subjected to thermal denaturation (melting). The reconstituted nucleohistones were melted in a 1-cm quartz cuvette, in which the solution was heated from 30 to 96°C, and the change in D at 260 nm was recorded every 2°C after preliminary exposure for 5 min to the assigned temperature. The melting point of the nucleohistone (mp) was determined as the temperature corresponding to half of the hyperchromic effect obtained during their denaturation. To identify the transitional zone on the melting profile the derived melting curve was plotted. For this purpose the temperature derivative of D was found by the equation

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

The transitional zone between the melting phases corresponded to the temperature at which the temperature derivative D was minimal.

EXPERIMENTAL RESULTS

Values of the stoichiometric ratio of histone/DNA for intact and estrogen-treated reconstituted nucleohistones in a concentration of 10^{-5} M did not change the protein/DNA ratio by weight for any type of individual nucleohistone. Essentially estrone, 17β-estradiol, and estriol, in the same concentration, did not affect the degree of binding of the complex of histones F_{2a}, F_{2b}, and F_3 with DNA in total nucleohistone. (The term "total nucleohistone" is applied to the nucleoprotein reconstituted from DNA and an equimolar complex of histones.) Such nucleohistone has been shown to simulate the structure of native DNP [11].

However, despite the absence of stoichiometric changes in the nucleohistones treated with estrogens the possibility cannot be ruled out that these steroids cause conformational changes in nucleoproteins. Criteria of the secondary structure of nucleohistones are the parameters of their thermal denaturation, i.e., mp and hyperchromic effect. The addition of histones to DNA in solution leads to the formation of DNP complexes that are more resistant to thermal denaturation than the nucleic acid. This is expressed as the appearance of a second phase on the melting curve, the melting point of which is much higher than that of free DNA. The melting profile of total nucleohistone consisted of a three-phase curve, in agreement with data obtained by other workers [10, 14]. Treatment of all types of nucleohistones studied in these experiments with estrogens caused no change in the corresponding melting points. However, treatment with hormones (esterone, 17β-estradiol, or estriol) changed the distribution of the total hyperchromic effect among the phases of melting of the individual nucleohistones F_{2a}, F_{2b}, and F_3 and total histone. This phenomenon was expressed as an increase in the hyperchromic effect of the first phase of melting and a corresponding decrease in the hyperchromic effect of the remaining phases (Table 2). The magnitude of this redistribution of the total hyperchromic effect varied from 31% (for nucleohistone F_{2a} treated with estradiol) to 11% (for nucleohistone F_{2b} treated with estriol). After treatment with estradiol or estrone the fraction of the hyperchromic effect corresponding to the first phase of melting of total nucleohistone was increased by 21% compared with the fraction of the hyperchromic effect of the second and third phases. By contrast with the remaining types of reconstituted nucleohistones, the profile of thermal denaturation of nucleohistone F_1, treated with estrogens, was indistinguishable from the control.

The changes in the thermal denaturation profile of total nucleohistone and of the partial nucleohistones F_{2a}, F_{2b}, and F_3 produced by estrogens, as described above, may be due, it can tentatively be suggested, to hormone-induced modification of interaction between the components of the nucleoprotein. One result of this modification is an increase in the total length of the free segments of DNA in the nucleohistone, which can take place during estrogen-dependent derepression of the genome of target cells. Interaction of histones F_{2a}, F_{2b}, and F_3 with one another in the composition of DNP has been shown to determine the subunit structure of the nucleohistone [6]. According to the subunit model of nucleohistone structure, DNA exists in the subunits as a superhelix [12]. It may be that the estrogen, with its increased affinity for hydrophobic regions, breaks the

weak histone–histone bonds (which are hydrophobic in nature) and so destabilizes the DNA superhelix. The absence of effects of estrogens during thermal denaturation of partially reconstituted nucleohistone F_1 confirms this hypothesis. Some workers have expressed the opinion that the lysine-rich F_1 histones do not take part in the formation of the subunit structure of DNP [4, 13].

LITERATURE CITED

1. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971), p. 147.
2. I. A. Lavrinenko, R. I. Salganik, and T. M. Morozova, Molekul. Biol., No. 1, 20 (1972).
3. R. E. Buller and B. W. O'Malley, Biochem. Pharmacol., 25, 1 (1976).
4. J. L. Demangeat, J. Chambron, and H. Benoit, Biochimie, 57, 845 (1975).
5. E. W. Johns, Biochem. J., 105, 611 (1967).
6. R. Kornberg, Science, 184, 868 (1974).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. H. R. Maurer and R. Chalkley, J. Mol. Biol., 25, 431 (1967).
9. R. A. Reisfeld, H. J. Lewis, and D. E. Williams, Nature, 195, 281 (1962).
10. R. L. Rubin and E. N. Moundrianakis, J. Mol. Biol., 67, 361 (1972).
11. R. L. Rubin and E. N. Moundrianakis, Biochemistry (Washington), 14, 1718 (1975).
12. C. Y. Saharabudhe and K. E. Van Holde, J. Biol. Chem., 249, 152 (1974).
13. A. J. Varshavsky, Biol. Zbl., 95, 301 (1976).
14. C. L. F. Woodcock and L.-L. Frado, Biochem. Biophys. Res. Commun., 66, 403 (1975).
15. K. R. Yamamoto and B. Alberts, J. Biol. Chem., 249, 7068 (1974).