A Trypsin Sensitive Site for the Action of Hydrocortisone on Calf Thymus Nuclei

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Summary: Hydrocortisone in physiological concentration caused a marked change in the circular dichroism spectrum of calf thymus nuclei. Selective hydrolysis of the histone components of the nuclei with trypsin inhibited with soy bean trypsin inhibitor rendered the treated nuclei unresponsive to the hormone. These results are interpreted as evidence for the involvement of histones in the interaction between hydrocortisone and calf thymus nuclei.

The observation that a number of steriod hormones, which affect RNA polymerase activity in tissues of several types, bind to histones *in vitro* (1, 2) suggests that the histone components of chromosomal material may be the site of action of these hormones. This suggestion is substantiated by the removal of a physico-chemical response of calf thymus nuclei to physiological concentrations of hydrocortisone upon selective hydrolysis of the histone components of the nuclei.

Methods. Nuclei were isolated from fresh calf thymus tissue by the proceedure of Alfrey et al. (3) and further purified by the method of Kodama and Tedeschi (4). The nuclei were washed into 0.01M Tris buffer (pH 8) containing 0.0033M $CaCl_2$ just prior to circular dichroism studies. Protein concentrations of the nuclear preparation were determined by the Lowry method and DNA concentrations by the Dische diphenylamine reaction. Circular dichroism (CD) measurements were made on a Cary Model 60 recording spectropolarimeter with CD attachment 6002. All measurements were made at ambient temperature in double cylindrical tandem cells, each compartment of 10mm. light path. The data are expressed in terms of specific ellipticity (ψ) based on the g/ml of the DNA and protein components of the nuclear suspensions.

Results: Figure 1 shows th CD spectra of calf thymus nuclei separated from and in the presence of 5 x 10^{-6}M hydrocortisone. The CD spectrum of calf thymus nuclei is characterized by two major CD bands at 225mμ and 207mμ and a small negative shoulder at 247mμ. In the presence of the hormone there is a marked decrease in the magnitude of the transition at 225mμ as well as a diminution in the shoulder at 247mμ while the CD band at 207mμ remains unchanged.

In order to determine whether this apparent hormone induced conformational change in the nuclei is dependent upon the presence of histones, the histone components of calf thymus nuclei were selectively hydrolyzed with tripsin inhibited by soy bean trypsin inhibitor. Trypsin inhibited in this manner has been shown by Allfrey et al. (5) to selectively hydrolyze the histone components of calf thymus nuclei without otherwise altering the nuclear structure. Figure 2 shows the CD spectra of calf thymus nuclei after treatment with inhibited trypsin (0.8mg of inhibitor per mg of trypsin) separated from and in the presence of 5 x 10^{-6}M hydrocortisone. The CD spectrum of trypsin treated calf thymus nuclei

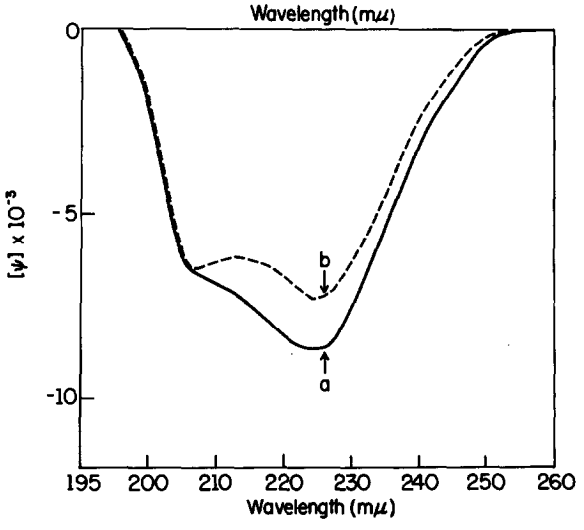

Figure 1: The circular dichroism spectra of calf thymus nuclei (4.4 x 10^{-3}mg/ml of DNA and 1.23 x 10^{-2}mg/ml of protein) and hydrocortisone (5 x 10^{-6}M) in 0.01M Tris buffer (pH 8) containing 0.0033M $CaCl_2$. a- hormone and nuclei in separate compartments of tandem cell. b- hormone and nuclei in same compartment of tandem cell.

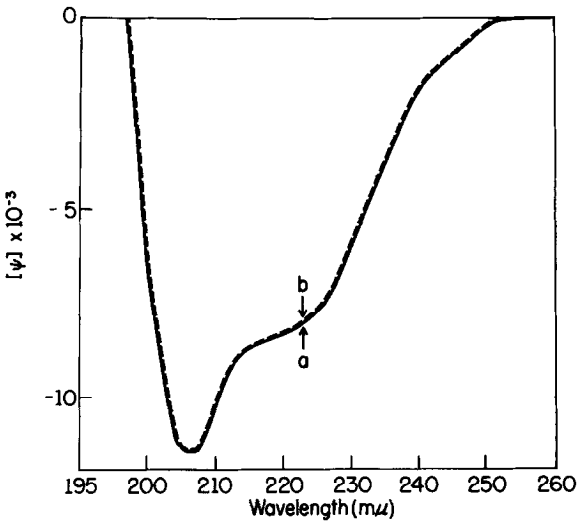

Figure 2: The circular dichroism spectra of calf thymus inhibited trypsin treated nuclei (1.0mg trypsin and 0.8mg soy bean trypsin inhibitor/50mg nuclei for 10 min. at 37°) (4.4×10^{-3}mg/ml of DNA and 1.23×10^{-2}mg/ml of protein) and hydrocortisone (5×10^{-6}M) in 0.01M Tris buffer (pH 8) containing 0.0033M $CaCl_2$. a- hormone and nuclei in separate compartments of tandem cell. b- hormone and nuclei in same compartment of tandem cell.

differs substantially from that of untreated nuclei. Although the 247mμ shoulder and the 225mμ band remain unchanged by tripsin treatment, the 207mμ band is increased 40%. The presence of 5×10^{-6} M hydrocortisone did not affect the CD spectrum of nuclei treated with inhibited trypsin. The inhibited tripsin treated nuclei appeared intact by phase contrast microscopy.

Discussion: Since the contribution of 5×10^{-6} M hydrocortisone to the CD spectrum of nuclei and hormone is negligible, the observed changes in the spectrum upon mixing hormone with nuclei (Fig. 1) appear to be the result of changes in the nuclei. These changes are primarily in the 225mμ band and most likely represent changes in the n-π^* transition which usually occurs in simple helical polypeptides at 222mμ. This shift to 225mμ may represent the contribution from the DNA spectrum with its maximum negative ellipticity at 247mμ. The observed changes in the n-π^* transition of calf thymus nuclei elicited by hydrocortisone most likely represent a decrease in the helical content of the nuclear proteins.

Treatment of calf thymus nuclei with inhibited trypsin caused a marked

increase in the ellipticity of the 207mμ band of the nuclei CD spectrum (Fig. 2) indicating some changes in the structural characteristics of the nuclear proteins due to trypsinization. Since the increase in ellipticity at 207mμ was not associated with a change in the 225mμ transition it is difficult to interpret the nature of these changes. After trypsinization, hydrocortisone no longer elicits a change in the CD spectrum of the treated nuclei (Fig. 2). Since treatment with tripsin in the presence of inhibitor for short periods of time (10 min.) results in the selective hydrolysis (5) of the histone components of the nuclei, it would appear that the major difference between inhibited trypsin treated and untreated nuclei is the presence of intact histones. The lack of response to hydrocortisone shown by inhibited trypsin treated nuclei strongly suggests that histones play a key role in the mechanism of the hormonal purturbation of the nuclei. Since the hormonal concentration used in these experiments is equal to that used by Beato et al. to cause changes in the RNA polymerase activity of liver nuclei (6) it seems likely that the conformational perturbations observed in response to hormone in this study may be directly related to hormonal control of the transcription process.

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