STRUCTURAL SPECIFICITY OF THE STEROIDS INTERACTING WITH CALF THYMUS HISTONES

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Intensive studies are being undertaken by various investigators to elucidate the mechanism of action of hormones. It has been postulated that steroids might act by uncovering gene expression (Karlson, 1965). Since histones are associated with DNA and inhibit DNA-dependent RNA polymerase (Stedman and Stedman, 1960; Huang and Bonner, 1962; Allfrey, Littau and Mirsky, 1963) steroids might act by interacting with histones. Sluyser (1966) reported that cortisol was bound with the lysine-poor fraction of calf and rat liver histones.

We have observed that steroids do interact with arginine-rich fraction of calf thymus histones (Sunaga and Koide, 1966). The interaction was determined by measuring the specific activities of labeled steroids bound with calf thymus histones upon gel filtration on Sephadex G-25. The purpose of this study was to determine what functional groups on the steroid nucleus influence this interaction.

MATERIALS AND METHODS

Arginine-rich histone (type IV) from calf thymus was purchased from Sigma Chemical Co., St. Louis, U.S.A. Tritium-labeled steroids and their specific activities are listed in Table I. They were purchased from New England Nuclear Corp., Boston, U.S.A. Glass fiber filter, Grade 934AH, 2.1 cm was

TABLE I

Names and Specific Activities of Steroids Used

Trivial Names	Chemical Names	Specific Activities: C/mmole
Tetrahydrocortisone	5β-pregnane-3α, 17α, 21-triol-11, 20-dione-1, 2-^{3}H	34.4
Cortisone	4-pregnene-17α, 21-diol-3, 11, 20-trione-1, 2-^{3}H	34.4
Cortisol	4-pregnene-11β, 17α, 21-triol-3, 20-dione-1, 2-^{3}H	10.0
Tetrahydrocortisol	5β-pregnane-3α, 11β, 17α, 21-tetrol-20-one-1, 2-^{3}H	18.2
Corticosterone	4-pregnene-11β, 21-diol-3, 20-dione-1, 2-^{3}H	33.9
17α-hydroxy-11-deoxycorticosterone	4-pregnene-17α, 21-diol-3, 20-dione-1, 2-^{3}H	2.0
11-deoxycorticosterone	4-pregnen -21-ol-3, 20-dione-1, 2-^{3}H	23.2
17α-hydroxyprogesterone	4-pregnen - 17α-ol-3, 20-dione-7α-^{3}H	9.9
17α-hydroxypregnenolone	5-pregnene-3β, 17α-diol-20-one-7α-^{3}H	9.9
Testosterone	4-androsten-17β-ol-3-one-7α-^{3}H	46.5
Progesterone	4-pregnene-3, 20-dione-7α-^{3}H	10
Aldosterone	4-pregnen-18-al-11β, 21-diol-3, 20-dione-1, 2-^{3}H	32.2
17β-estradiol	1, 3, 5 (10)-estratriene-3, 17β-diol-6, 7-^{3}H	25

purchased from Scientific Glass Apparatus Co., Clifton, N.J. U.S.A. Phosphor for scintillation counting was prepared by diluting 42 ml of liquifluor (Pilot Chemicals, Inc., Mass. U.S.A.) with a liter of toluene. A solution of histones in 0.2 M potassium phosphate buffer, pH 7.3, at a concentration of 6 mg/ml was prepared immediately before use. A solution of labeled steroid (μC/0.1 ml of 0.2 M phosphate buffer, pH 7.3) was prepared, allowed to stand for 24 to 96 hours at room temperature and stored at 0-4^{o}. When the steroid solutions were re-tested after 3 months, their ability to interact with histones remained essentially unchanged. An aliquot of steroid solution (0.1 ml) was mixed with 0.5 ml of the histone solution and kept at room temperature for 30 min. The mixture was placed on a Sephadex G-25 column (0.8 x 32 cm) and eluted with 0.2 M phosphate buffer, pH 7.3. The filtration procedure was performed at 0-5^{o}. Several samples of histones were run on the column until a recovery of greater than 90% of protein was obtained before it was used in the study. Fractions were collected at a rate of 0.5 ml/min. Protein was determined by the biuret method (Gornall, Bardawill and David, 1949). Radioactivity was determined by placing 50μl of each fraction on a glass fiber filter. The filter was placed in a vial and dried at 90^{o} for 30 min. To each vial 10 ml of phosphor in toluene were added and the samples were counted in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003.

The amount of steroids bound to histones was calculated from the specific activities (cpm/mg of protein). The average counting efficiency for tritium was found to be 18% which was used in the calculation. It was observed that the amount of steroids bound to histones increased with increasing increments of steroids used in the incubation system. The relationship was almost linear at the concentrations used in this study which ranged from 7.1 to 167 $\mu\mu$moles/mg of protein. Since the binding capacity varies with the concentration of steroids to histones, equal amounts of steroids were used (100 $\mu\mu$moles/mg of histones).

Table II

Binding of Steroids to Calf Thymus Histones.

Steroids	μμmoles/mg of protein
Tetrahydrocortisone	11.1
Cortisone	5.6
Cortisol	2.9
Tetrahydrocortisol	2.9
Corticosterone	0.9
17α-hydroxy-11-deoxycorticosterone	0.9
11-deoxycorticosterone	0.8
17α-hydroxyprogesterone	0.4
17α-hydroxypregnenolone	0.4
Testosterone	0.3
Progesterone	0.3
Aldosterone	0.2
17β-estradiol	0.2

The values are averages of 3 to 5 separate experiments. The values were calculated from experiments utilizing 100 μμmoles of steroids per mg of histones.

RESULTS AND DISCUSSION

The binding of steroids with the arginine-rich fractions of calf thymus histones is shown in Table II. In general, the binding capacity was greatest with 17-hydroxycorticosteroids and lowest with testosterone, 17β-estradiol, aldosterone and progesterone.

The results of this study suggest that there are two sites on the steroid nucleus which participate in the interaction with histones. These sites are the 11-keto or 11β-hydroxyl and the dihydroxyacetone groups. Any modification or deletion of these groups appear to markedly decrease the interaction between the steroid and histone.

The variation in the binding of cortisone, cortisol and 17α-hydroxy-11-deoxycorticosterone (Table II) indicates that the interaction of the steroids with histones is dependent on the functional group at carbon 11. The binding was greatest with 11-keto derivatives, slightly less with 11β-hydroxyl and least with 11-deoxy derivatives. These results are further supported by the finding that the

binding of tetrahydrocortisone with histone was greater than that of tetrahydrocortisol (Table II).

The second site of interaction on the steroid molecule is the dihydroxyacetone group. Interaction of histone with this functional group may take place with the hydroxyl groups of C-17 and C-21. The 17-hydroxyl group may participate in the interaction since the binding of cortisol with histone markedly exceeded that of corticosterone (Table II). The specific activity of 17α-hydroxy-11-deoxycorticosterone bound to histones, however, was in the same low range as 11-deoxycorticosterone (Table II), suggesting that the influence of the 17α-hydroxyl on the interaction is minimal or insignificant in the absence of the 11β-hydroxyl or 11-keto group. The contribution of the 21-hydroxyl group to the interaction was not assessed since 11β, 17α-dihydroxyprogesterone was not available. The binding of 17α-hydroxy-11-deoxycorticosterone with histone was slightly greater than that of 17α-hydroxyprogesterone (Table II), indicating that the influence of the 21-hydroxyl group on the interaction is slight in the absence of the 11-hydroxyl group. Moreover, in the absence of the 11β- and 17α-hydroxyl groups, the contribution of the 21-hydroxyl to the interaction was minimal which was substantiated by the finding that the binding of 11-deoxycorticosterone with histones was slightly greater than that of progesterone (Table II). One can conclude from these results that the dihydroxyacetone group does influence the interaction to some degree. The binding is markedly reduced in the absence of the 11-keto or 11β-hydroxyl group.

Although aldosterone possesses 11β-hydroxyl, α, β-unsaturated 3-keto and α-ketol groups, the binding of aldosterone with histone was low. The anomalous low binding may be caused by the formation of a hemiacetal between the C-18 aldehyde and 11β-hydroxyl group which prevents the 11β-hydroxyl group from participating in the interaction. The low binding of aldosterone is consistent

with the proposition that in the absence or modification of the 11β- and 17α-hydroxyl groups the influence of the 21-hydroxyl group to the interaction is slight or minimal.

The participation of the α,β-unsaturated 3-keto group in the interaction is questionable since the binding of cortisone and cortisol with histones was equal to or less than that of the hydrogenated derivatives; tetrahydrocortisone and tetrahydrocortisol. Furthermore, other steroids with α,β-unsaturated 3-keto group such as progesterone, aldosterone and testosterone were poorly bound.

It is noteworthy that the binding of cortisone and cortisol with histones far exceeded that of aldosterone and 11-deoxycorticosterone, suggesting that glucocorticoids may mediate their actions by interacting with histones, whereas the mineralocorticoids probably do not.

On further inquiry into the nature of the binding phenomenon, we have observed that the steroid-histone interaction is influenced by changes in pH, buffer and temperature. It was further observed that the interval between the time of preparation of the steroid solutions and its incubation with histones influenced the interaction. The binding of cortisone, tetrahydrocortisone, cortisol and tetrahydrocortisol with histones increases with the duration of storage of the steroid solutions at room temperature. There was a minimal to slight increase in binding with the other compounds after 96 hours of storage. Preliminary study suggests that the storage effect may be due to complex formation of the steroids with phosphates. This problem is presently under investigation.

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