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Stokes Radius of Human Pituitary Hormones and Demonstration of Dissociation of Luteinizing Hormone*

Robert J. Ryan

ABSTRACT: A technical modification, namely the use of radioiodine-labeled proteins, has been used in the gel filtration technique for the determination of the Stokes radius of human pituitary follicle-stimulating hormone, luteinizing hormone, somatotrophic hormone, and thyroid-stimulating hormone. Data are presented to justify the use of this technical modification. The Stokes radii of these pituitary hormones were found to be 32.2 Å for follicle-stimulating hormone, 22.2 Å for somatotrophic hormone, and 27.5 Å for thyroid-stimulating hormone.

Two species of luteinizing hormone molecules were found, the larger having a Stokes radius of 30.2 Å and the smaller, 22.6 Å. The molecular weights of these species of luteinizing hormone were estimated to be 28,000 and 14,400.

Porath and Flodin (1959) described the technique of separating materials of different molecular sizes by passing a solution of the substances through a bed of porous gel with solvent phases inside and outside of the gel grains. Whitaker (1963) and Andrews (1964) proposed that this technique could be used for estimating the molecular weight of unknown proteins by comparing their elution volumes with those of proteins of known molecular weight. However, it became apparent (Laurent and Killander, 1964; Siegel and Monty, 1966; Squire, 1964) that this estimation of molecular weight was valid only when the unknown protein had a partial specific volume and a frictional ratio similar to those of the standards used. Evidence was provided that the elution position of a protein during gel filtration was related to the Stokes radius of the molecule (an index of its size) rather than to its molecular weight.

The present study was undertaken to determine the Stokes radius of human pituitary luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and somatotrophic hormone. These measurements were performed with radioiodinated preparations of the hormones and the validity of this technical modification was established. In addition evidence is presented for the existence of associated and dissociated forms of human luteinizing hormone.

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TABLE I: Stokes Radii and K_{av} of Standard Proteins.

Standard Protein	Stokes Radius (Å)	K_{av} ± Std Error (n)	
		Buffer A[a]	Buffer B[b]
Human γ-globulin	55.5	0.024 ± 0.002 (3)	
Bovine serum albumin	34.9–36.1	0.136 ± 0.009 (14)	0.1307 ± 0.0032 (7)
Ovalbumin	27.3	0.230 ± 0.036 (3)	0.206 (2)
Ovomucoid	26.8–27.8	0.274 (1)	0.213 (1)
Chymotrypsinogen	22.4	0.363 ± 0.027 (10)	0.345 ± 0.007 (7)
Myoglobin	18.8–20.7	0.394 ± 0.001 (4)	0.384 ± 0.002 (3)
Ribonuclease	19.2	0.404 ± 0.002 (3)	0.415 ± 0.014 (3)
Cytochrome *c*	16.4	0.510 ± 0.034 (9)	

[a] 0.2 M NaCl–0.01 M phosphate (pH 7.5). [b] 1.0 M NaCl–0.01 M phosphate (pH 7.5).

TABLE II: Summary of Hormone Preparations.

Preparation	Biologic Potency		Method of Preparation
	Follicle-Stimulating Hormone (U/mg)[a]	Luteinizing Hormone (U/mg)[b]	
RR 5765B	100	0.08	Ryan (1968)
LER 869-2[c]	116		Reichert *et al.* (1968)
LER 960[c]		1.56	Reichert *et al.* (1968)
RR 41965B1BA2	<0.06	3.35	Ryan (1968)
AP DEAE II-2[c]	0.08	4.10	Parlow's modification of Hartree *et al.* (1964)
RR 41267B2BA2	0.05	3.03	Ryan (1968)

[a] NIH follicle-stimulating hormone S1 units/mg. [b] NIH luteinizing hormone S1 units/mg. [c] These preparations were obtained from the National Pituitary Agency, Baltimore, Md. All are considered to be sufficiently pure to be used for radioimmunoassays.

Material and Methods

Gel Filtration Procedure. Sephadex G-100 (particle size, 40–120 μ; water regain, 10 g/g; bed volume, 15–20 ml/g; and density, 0.736) was used. The gel was soaked in buffer for 48 hr prior to use and the fines were removed by frequently aspirating the buffer and resuspending the settled gel beads in fresh buffer. Two buffers were used: buffer A = 0.2 M NaCl–0.01 M phosphate (pH 7.5) and buffer B = 1.0 M NaCl–0.01 M phosphate (pH 7.5).

The hydrated gel was poured into a column 2.5 cm in diameter. In individual experiments the bed height varied from 89 to 93.5 cm. The column was kept at 10° by a water jacket. The column was developed by upward flow provided by a constant infusion pump. The flow rate was constant during any one experiment but varied from 10 to 20 ml per hr from one experiment to another. The eluates were collected in 2.1-ml portions by a fraction collector and were monitored for absorbancy at 225 mμ. Where indicated, radioactivity was measured with a Nuclear-Chicago Autogamma system. In some experiments, follicle-stimulating hormone and luteinizing hormone were measured in the eluates by specific radioimmunoassays previously described by Faiman and Ryan (1967a,b).

The fraction of the volume of the gel bed available for diffusion of each protein or hormone was calculated by using the equation of Laurent and Killander (1964)

$$K_{av} = \frac{V_e - V_o}{V_t - V_o} \quad (1)$$

in which V_e = elution volume of the specific protein, measured as the elution peak as described by Andrews (1964); V_o = void volume of the column; and V_t = total volume of the column.

The void volume of the column, V_o, was taken to be the elution volume of Blue Dextran 2000.

In each experiment the K_{av} of two or more standard proteins and a hormone preparation were determined. The Stokes radii of the standard proteins were then plotted arithmetically against $(-\log K_{av})^{1/2}$. This relationship, proposed by Laurent and Killander (1964), was demonstrated to be a linear function by Siegel and Monty (1966). Calculation of $(-\log K_{av})^{1/2}$ for the hormone then permitted the Stokes radius to be read from the standard curve.

Standard Protein Preparations. The proteins used as standards for calibrating the gel filtration columns were all obtained from Mann Research Laboratories. They

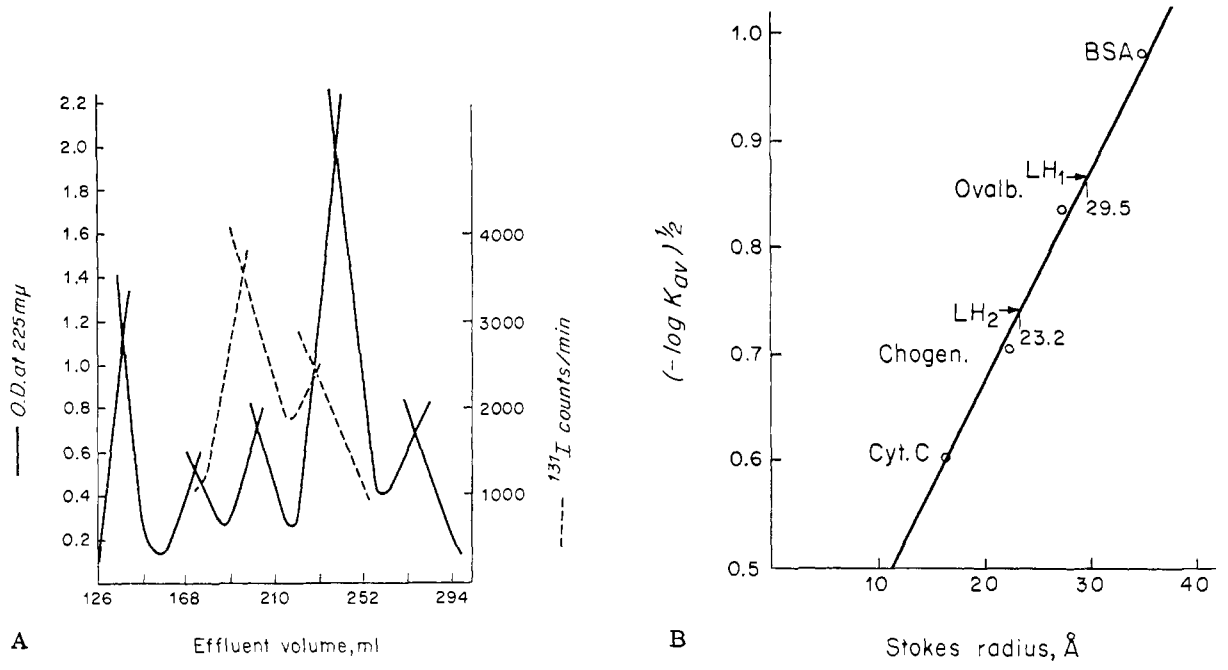

FIGURE 1: Elution patterns and Stokes radii. (A) Elution pattern of typical gel filtration column (column volume, 459 ml). Elution volumes of standard materials (solid lines), reading from left to right, were: Blue Dextran, 136.9 ml; bovine serum albumin, 172.2 ml; ovalbumin, 201.6 ml; chymotrypsinogen, 239.8 ml; and cytochrome *c*, 277.2 ml. Dashed line represents ^{131}I-labeled luteinizing hormone, RR 41965B1BA2, with two elution peaks, at 195.3 and 228.9 ml. (B) Plot of Stokes radii in Ångströms against $(-\log K_{av})^{1/2}$ for materials shown in part A.

were used in quantities of 1–4 mg. Values for the Stokes radii of these proteins (Table I) were taken from Laurent and Killander (1964) except for ovomucoid. The Stokes radius of ovomucoid was calculated from the diffusion constants quoted by Melamed (1966) by using

$$a = kT/6\pi\eta D \qquad (2)$$

in which a = Stokes radius in Å, k = Boltzmann constant, T = absolute temperature, η = viscosity of the medium, and D = diffusion constant.

Pituitary Hormone Preparations. The various preparations of human follicle-stimulating hormone and luteinizing hormone used in these studies are given in Table II along with their potencies and their sources. Most of these were studied by observing the elution position of their radioiodinated derivatives. The elution position of two preparations (follicle-stimulating hormone RR 5765B and luteinizing hormone RR 41967B1BA2), not labeled with ^{131}I, were localized by specific radioimmunoassays. These latter materials were applied to the gel filtration columns in 10-μg quantities.

The elution position of one highly purified preparation of luteinizing hormone (RR 41267B2BA2), not labeled with ^{131}I, was determined by $OD_{225\ m\mu}$ when 4 mg was applied to the column. This preparation was also studied after incubation for 5 hr at room temperature with 4.0 M guanidine hydrochloride at pH 6.5 (Mann, Ultra Pure) (5 mg of protein in 0.2 ml). The mixture of guanidine and protein was applied directly to the column.

Immunochemical grade preparations of human thyroid-stimulating hormone and somatotrophic hormone were obtained from the National Pituitary Agency. The human thyroid-stimulating hormone preparation, which is known to contain human luteinizing hormone, was adsorbed with an antiserum to human luteinizing hormone, after labeling with ^{131}I, to remove this contaminant.

Radioiodination Procedure. Protein (1–2 μg) was labeled with ^{131}I by the method of Greenwood *et al.* (1963). Specific activities of 90–500 μCi/μg were obtained. ^{131}I-labeled protein (2–5 ng) was applied to the gel filtration columns.

Results

The elution pattern of a typical column is illustrated in Figure 1A. The plot of $(-\log K_{av})^{1/2}$ *vs.* Stokes radius for the same experiment is illustrated in Figure 1B.

The Stokes radius of chymotrypsinogen labeled with ^{131}I to a specific activity of 90 μCi/μg was 22.0 and 22.7 Å in two separate determinations. The mean value (22.35 Å) agrees very well with the reported value of 22.4 Å for the unlabeled protein. In addition, bovine serum albumin, labeled with ^{131}I to a specific activity of 590 μCi/μg, gave a Stokes radius of 36.4 Å, a value which agrees well with the reported values of 34.9–36.1 Å for the unlabeled protein (Table I).

Stokes Radius of Human Follicle-Stimulating Hormone. Only one species of follicle-stimulating hormone was seen during these studies. As presented in Table III, follicle-stimulating hormone 5765B labeled with ^{131}I had a Stokes radius of 32.4 Å when examined in buffer A and 31.5 Å when examined in buffer B (difference is not statistically significant). The Stokes radius

TABLE III: Stokes Radii of Human Pituitary Hormones.

Hormone	Labeled with ^{131}I	Stokes Radius (Å) ± Std Error (*n*)	
		Buffer A	Buffer B
Follicle-stimulating hormone			
RR 5765B	Yes	32.4 ± 0.3 (5)	31.5 ± 0.4 (3)
RR 5765B	No	31.5 (1)	
LER 869-2	Yes	33.7 (1)	
Luteinizing hormone (larger species)			
RR 41965B1BA2	Yes	30.4 ± 0.5 (4)	29.6 ± 0.2 (3)
RR 41965B1BA2	No	31.0 (1)	29.9 (1)
LER 960	Yes	30.8 (2)	
RR 41267B2BA2	No	29.7 (2)	
Luteinizing hormone (smaller species)			
RR 41965B1BA2	Yes	22.3 ± 0.8 (4)	23.2 ± 0.4 (3)
RR 41267B2BA2	No[a]	22.0 (1)	
Thyroid-stimulating hormone	Yes	28.0 (1)	
	Yes	27.0 (1)	
Somatotrophic hormone	Yes	22.8 (1)	
	Yes	21.6 (1)	

[a] Treated with 4.0 M guanidine hydrochloride.

of an ^{131}I-labeled preparation of follicle-stimulating hormone purified by a different procedure (LER 869-2) and of a nonradioactive preparation of follicle-stimulating hormone was 33.7 and 31.5 Å, respectively. Because no significant difference was noted between labeled and unlabeled preparations, between buffers, or between the various preparations of follicle-stimulating hormone, an over-all average was calculated, giving the value 32.2 Å.

Stokes Radius of Human Luteinizing Hormone. Two species of luteinizing hormone were seen in studies of ^{131}I-labeled luteinizing hormone with three different preparations (RR 41965B1BA2, LER-960, and AP-DEAE-II-2). This is illustrated in Figure 1. However, the distribution of the two species varied with the preparation and the buffer. With preparation RR 41965B1BA2, in buffer A 60–70% emerged as the larger species (30.4 Å) and 30–40% as the smaller species (22.3 Å); in buffer B, the distribution was reversed but the Stokes radii of the two species were similar to the values in buffer A (29.6 and 23.2 Å). Preparation AP DEAE-II-2 was also found to have two species, in approximately equal quantities, when examined in buffer A but because of technical error the Stokes radii for this preparation could not be calculated. Preparation LER-960 was examined only in buffer A. Ninety to ninety-five per cent of the material emerged as the larger molecular species (Stokes radius, 30.8 Å). The Stokes radius of the minor portion emerging as the smaller species could not be determined accurately.

When examined without ^{131}I labeling, preparation RR 41965B1BA2 showed only one species when the eluates were studied by radioimmunoassay. This species had a Stokes radius of 31.0 Å in buffer A and 29.9 Å in buffer B. When 10 μg of unlabeled hormone was added to 2 ng of labeled hormone and subjected to gel filtration in buffer B, virtually all of the labeled hormone emerged as the larger molecular species.

To ascertain whether unlabeled hormone could be dissociated, 4–5 mg of preparation RR 41267B2BA2 was subjected to gel filtration before and after treatment with 4 M guanidine hydrochloride. The results are illustrated in Figure 2. The native material emerged from the column as a single peak with a Stokes radius of 29.9 Å (29.5 Å in a second determination). After treatment with 4 M guanidine hydrochloride, the major portion of the material emerged at a position corresponding to a Stokes radius of 22 Å, and a minor shoulder was seen corresponding to a larger species.

Since it did not appear from the data in Table III that the Stokes radii of the two species were significantly affected by the buffer, the preparation, or the presence of an ^{131}I label, over-all averages were calculated. The larger species of luteinizing hormone had an average Stokes radius of 30.2 Å, a value significantly ($P < 0.02$) smaller than the radius of follicle-stimulating hormone. The smaller species of luteinizing hormone had an average Stokes radius of 22.6 Å.

Stokes Radii of Human Somatotrophic Hormone and Thyroid-Stimulating Hormone. Both of these materials behaved during gel filtration as single species of molecules with Stokes radii of 22.2 Å for somatotrophic hormone and 27.5 Å for thyroid-stimulating hormone.

Discussion

The use of radioiodinated proteins for the determination of Stokes radius has advantages and possible disadvantages. It permits easy detection of the material,

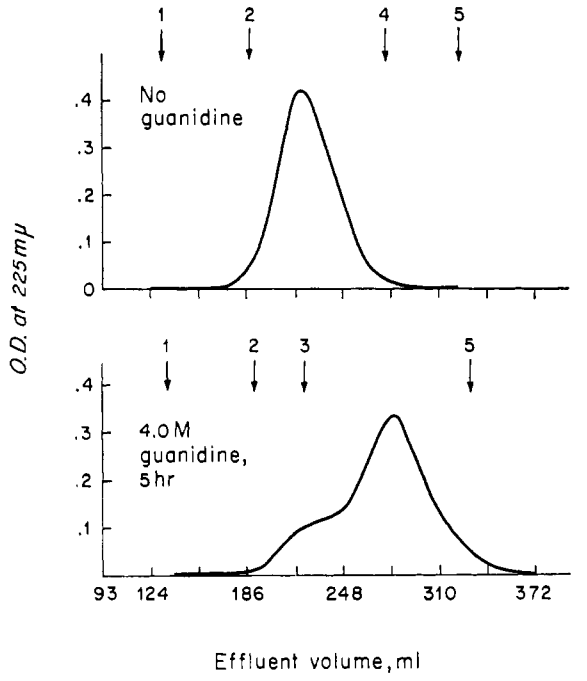

FIGURE 2: Elution pattern of luteinizing hormone (RR 41267B2BA2) before and after treatment with 4.0 M guanidine hydrochloride. Numbered arrows indicate elution peaks of standard materials: 1 = Blue Dextran; 2 = bovine serum albumin; 3 = ovomucoid; 4 = chymotrypsinogen; and 5 = cytochrome *c*.

it conserves material (which, with human protein hormones, is often available only in limited supply), and it allows working at conditions approaching zero concentration.

The disadvantages seem to be theoretical because the data presented herein suggest that there is no significant observable difference between the Stokes radii calculated from labeled and unlabeled proteins. Theoretically, one might expect the added iodine to increase the Stokes radius of the protein molecule. Under the conditions of iodination with carrier-free tracer used in this study, it can be calculated that less than 1 mole of iodine is added per mole of protein. One may visualize this iodine as being entirely within, partially within, or almost entirely outside the radius of the protein. Let us assume the third case. The radius of an iodide ion (I^-) has been reported as 2.15 Å[1] and a hydrogen ion as 1.1 Å (Pauling, 1960). The bond length between carbon and hydrogen is 1.06 Å while that between an aromatic carbon and iodine is 2.05 Å (Kennard, 1963). Thus, the maximal increase in radius from the substitution of one atom of iodine for one atom of hydrogen on the protein would be 2.04 Å ([2.15 + 2.05] − [1.06 + 1.1]). For the pituitary hormones reported herein, this would result in a maximal overestimation of the true Stokes radius by 6–9%.

A second theoretical disadvantage is that structural changes, resulting in a change in radius, may be induced in the protein by the oxidation or reduction reactions involved in the iodination procedure or by radiation damage. The evidence does not bear this out. No noticeable change in the Stokes radius of follicle-stimulating hormone was noticed whether the specific activity was 120 or 500 μCi/μg or whether the material was studied 1 day or 2 weeks after iodination. Labeled follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone were found to bind specifically to antibody both before and after gel filtration (R. J. Ryan, unpublished data).

Data already published concerning the gel filtration behavior of several human pituitary hormones were recalculated to obtain Stokes' radii (Table IV). These values are in good agreement with the results of the present study.

In the present study it was not possible to find evidence of more than one species of human follicle-stimulating hormone, although previous reports suggested that two or more species of differing size might exist (Czygan *et al.*, 1965; Gray, 1967; Ryan, 1968).[2]

The finding of two species of human luteinizing hormone was not a surprise. It was previously reported (Ryan, 1968) that human luteinizing hormone had $s_{20,w}$ values of 1.57 S at pH 1.2 and 2.28 S at pH 7.4. A similar observation was made for ovine luteinizing hormone by Li and Starman (1964). Recently, De la Llosa and Jutisz (1968) demonstrated by gel filtration and ultracentrifugal techniques that ovine luteinizing hormone could be dissociated by acid or guanidine; the larger species had a Stokes radius of 30 Å and a $s_{20,w}$ of 2.37 S and the smaller species, 24 Å and 1.54 S, respectively. Previous studies have indicated that human and ovine luteinizing hormone have similar, although not identical, chemical compositions (Kathan *et al.*, 1967) and similar elution volumes during gel filtration (Reichert, 1965).

Evidence for the interconvertibility of the two species of human luteinizing hormone reported herein is not as yet available. When the larger species was isolated and rerun through a column, the smaller species was again generated. However, when the smaller species was isolated, concentrated, allowed to stand for 24 hr at 4°, and then rerun through the column, the larger species was not regenerated. Although both the larger and smaller species of luteinizing hormone are recognized by radioimmunoassay, I cannot at this time state their relative potencies. However, when obtained after incubation with guanidine, the smaller species has less than 10% of the biologic potency of the larger species (R. J. Ryan, unpublished data). De la Llosa and Jutisz (1968) noted that the dissociated form of ovine luteinizing hormone

[1] This is the value for the crystal radius of $^{127}I^-$ and is probably not accurate for ^{131}I bound covalently to an aromatic carbon and which prior to covalent bonding would have a charge of 1+. Iodine 5+ and 7+ both have radii less than 1 Å and therefore one would expect the 1+ ion to be smaller than the 1− ion.

[2] Since preparation of this manuscript, it has been found that human follicle-stimulating hormone can be dissociated in the presence of guanidine (R. J. Ryan, N. S. Jiang, and M. S. Hanlon, unpublished data).

TABLE IV: Stokes Radii Calculated from Published Elution Volumes.

Preparation	Calcd Stokes' Radius (Å)	Reference
Human luteinizing hormone	30.0	Reichert (1965)
Human luteinizing hormone	29.2	Reichert *et al.* (1968)
Human luteinizing hormone	28.7	Reichert *et al.* (1968)
Human follicle-stimulating hormone	31.6	Reichert *et al.* (1968)
Human follicle-stimulating hormone	32.1	Reichert *et al.* (1968)

lost most of its biologic activity but, by concentration and incubation, they were able to regenerate the associated form and the biologic activity. It appears to be more difficult to dissociate human luteinizing hormone than ovine luteinizing hormone and it may therefore be more difficult to regenerate the associated form of the human hormone. For example, De la Llosa and Jutisz (1968) noted that incubation in 2 M guanidine hydrochloride for 2 hr dissociated 90% of ovine luteinizing hormone. These same conditions dissociated only 10% of human luteinizing hormone (R. J. Ryan, unpublished data). Hartree (1968) has noted that more acidic conditions were required to dissociate human luteinizing hormone than ovine luteinizing hormone.

The relationship between Stokes radius, a, sedimentation constant, s, and molecular weight, M, may be expressed as

$$M = \frac{6\pi\eta Nas}{1 - \bar{V}\rho} \qquad (3)$$

in which η = viscosity, N = Avogadro number, and $1 - \bar{V}\rho$ = partial specific volume of the protein.

With an assumed partial specific volume of 0.72, the larger species of human luteinizing hormone (s = 2.28; a = 30.18) has a molecular weight of 27,960 and the smaller species (s = 1.57; a = 22.6) has a molecular weight of 14,400. The molecular weight of the larger species should be compared with the value of 26,000 reported by Squire *et al.* (1962) on the basis of sedimentation-equilibrium analysis.

If the assumption is made that both the larger and smaller species of luteinizing hormones are perfect spheres, then a discrepancy appears between calculated and observed radii. For example, if the larger species of luteinizing hormone had a radius of 30.2 Å, then the smaller species would have a calculated value of 24 Å rather than the observed value of 22.6 Å. There are three possible explanations for this discrepancy: (1) the model of a perfect sphere is incorrect; (2) the difference between calculated and observed values is due to experimental error; and (3) a conformational change has occurred which has affected one species more than the other.

Ward *et al.* (1966) and Papkoff and Samy (1967) obtained two subunits of ovine luteinizing hormone with different chemical compositions. The relationship of the subunits reported by these workers to the two species of luteinizing hormone noted herein remains to be elucidated.

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Regulation of Rat Testis Steroid Sulfatase. A Kinetic Study*

Albert D. Notation and Frank Ungar

ABSTRACT: A variety of unconjugated steroids were found to inhibit the *in vitro* cleavage of dehydroepiandrosterone sulfate by rat testis tissue. The glucosiduronates of cortisol and cortisone had virtually no effect on the cleavage of dehydroepiandrosterone sulfate whereas the liberation of labeled dehydroepiandrosterone was efficiently suppressed in the presence of pregnenolone sulfate. The emerging trend of effective inhibition involves testosterone, estradiol, and possible biochemical precursors including pregnenolone. The inhibition constants of dehydroepiandrosterone ($K_i = 7.5 \times 10^{-6}$ M) and of testosterone ($K_i = 7.0 \times 10^{-6}$ M) for dehydroepiandrosterone sulfate cleavage ($K_m = 1 \times 10^{-5}$ M) were comparable with the inhibition constants of pregnenolone ($K_i = 8 \times 10^{-6}$ M) and of testosterone ($K_i = 8 \times 10^{-6}$ M) for pregnenolone sulfate cleavage ($K_m = 1 \times 10^{-5}$ M). Cholesteryl sulfate cleavage ($K_m = 5 \times 10^{-5}$ M) was inhibited by cholesterol ($K_i = 12 \times 10^{-6}$ M), testosterone ($K_i = 7.0 \times 10^{-6}$ M), and pregnenolone sulfate ($K_i = 4 \times 10^{-6}$ M). The kinetic data in all cases are compatible with competitive inhibition. Whatever the significance of the role of steroid sulfates in steroid hormone production might be, the present studies indicate that an effective control exists for the regulation of hormone synthesis *via* this pathway.

An initial report described how either exogenous dehydroepiandrosterone[1] or testosterone could inhibit the *in vitro* cleavage of DHEA-SO_4 in rat testis tissue. In contrast to this, the conversion of DHEA into its sulfate appeared to be independent of the amount of DHEA-SO_4 present. In spite of the low interconversion of DHEA and its sulfate, this inhibitory effect provides some basis for a possible regulation of the sulfate contribution to steroid sex hormone biosynthesis (Notation and Ungar, 1968). This report describes other steroid compounds which can inhibit the *in vitro* cleavage of DHEA-SO_4. Kinetic comparisons of the cleavages and selected inhibitions of the cleavages *in vitro* of DHEA-SO_4 pregnenolone sulfate, and cholesteryl sulfate, respectively, are also presented.

Methods and Materials

Dehydroepiandrosterone-7α-^{3}H sulfate, ammonium salt (labeled DHEA-SO_4) (15 Ci/mmole), pregnenolone-7α-^{3}H sulfate, ammonium salt (labeled-Preg-SO_4) (12.2 Ci/mmole), and cholesteryl-7α-^{3}H sulfate, ammonium salt (5.2 Ci/mmole) were obtained from the New England Nuclear Corp., Boston. Unlabeled unconjugated steroids were obtained from Mann Research Laboratories. Unlabeled DHEA-SO_4-NH_4, Preg-SO_4-NH_4, and cholesteryl-SO_4-NH_4 were synthesized, solvents were prepared, and steroid purity was checked by methods previously described (Notation and Ungar, 1968). Cortisol-21-glucosiduronic acid and cortisone-21-glycosiduronic acid were obtained from Dr. V. X. Mattox.

Preparation of Rat Testis Tissue Homogenate. Sprague–Dawley rats (180–200 g) were treated with human chorionic gonadotrophin for 3 days prior to sacrifice and homogenate of testis tissue was prepared as previously reported (Notation and Ungar, 1968). The buffer used in these latter experiments was a Krebs–Ringer 0.1 M bicarbonate buffer (pH 7.4) containing 200 mg of glucose/100 ml and 10 mg of fumaric acid/100 ml. Whole homogenates were used initially in the study of DHEA-SO_4 cleavage and subsequently compared with several particulate fractions which were initially obtained by removing the heavy tissue fragments from whole homogenates centrifuged at 4000*g* for 10 min.

Incubation and Extraction Procedures. Steroid substrates were dissolved in 10 μl of propylene glycol and the flasks (22-ml screw-top vials with foil-lined caps) were cooled on ice. Cold testis tissue homogenate (1.0 ml containing 100 mg of tissue) and a cofactor solution (50 μl) containing 100 μg each of ATP, β-DPN, and K_2SO_4 were added to each flask. Incubations were carried out in duplicate at 37° under an atmosphere of O_2–CO_2 (95:5) in a Dubnoff metabolic shaker. The incu-

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[1] The following trivial names are used: dehydroepiandrostrone (DHEA), 3β-hydroxy-5-androsten-17-one; pregnenolone (Preg), 3β-hydroxy-5-pregnen-20-one; progesterone, 4-pregnene-3,20-dione; testosterone, 17β-hydroxy-4-androsten-3-one; androsterone, 3α-hydroxy-5α-androstan-17-one; etiocholanolone, 3α-hydroxy-5β-androstan-17-one; estradiol, estra-1,3,5(10)-triene-3,17β-diol; cortisol, 11β,17α,21-hydroxy-4-pregnene-3,20-dione; cortisone, 17α,21-dihydroxy-4-pregnene-3,11,20-trione.