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ACTIVATION OF Δ^5-3-KETOSTEROID ISOMERASE OF BOVINE ADRENAL MICROSOMES BY SERUM ALBUMINS

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SUMMARY. The Δ^5-3-ketosteroid isomerase (EC 5.3.3.1) of bovine adrenal microsomes is activated as much as 10- to 20-fold by micromolar concentrations of bovine serum albumin. Comparable activations are observed with the serum albumins of 10 other mammalian species, but are not seen with ovalbumin or conalbumin. Evidence that the activation is attributable to the serum albumins, rather than to a small, firmly-bound ligand, is based on: (1) Failure to remove the stimulatory activity from the albumin by chloroform extraction, dialysis, or gel filtration; (2) Destruction of the activity by heating or by trypsin digestion; (3) Precipitation of the stimulatory activity of bovine serum albumin by specific antibody. Bovine serum albumin induces small decreases in the Michaelis constant for Δ^5-androstene-3,17-dione, but most of the activational effect reflects an increase in the maximum velocity. Low concentrations of Triton X-100, which are without effect on the isomerase activity, prevent the activation by bovine serum albumin.

INTRODUCTION

The conversion of dehydroepiandrosterone (3β-hydroxy-Δ^5-androsten-17-one) to Δ^4-androstene-3,17-dione and of pregnenolone to progesterone are key steps in the biosynthesis of steroid hormones. Each transformation involves a nicotinamide nucleotide-linked 3β-hydroxysteroid dehydrogenase (EC 1.1.1.145) and a Δ^5-3-ketosteroid isomerase (EC 5.3.3.1). The dehydrogenase-isomerase reaction sequence has been investigated extensively in steroidogenic tissues (1), and especially in the microsomal membrane fraction of bovine and rat adrenal cortex, which are rich sources of these enzymes. Several studies have dealt with the enhancement of these enzymatic activities by low molecular weight "activators" and by serum proteins. Bovine adrenal microsome isomerase activity is profoundly and specifically stimulated by low

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Abbreviations: BSA, bovine serum albumin; isomerase, Δ^5-3-ketosteroid isomerase.

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(0.1 - 0.5 μM) levels of NAD or NADH (2). This effect does not involve a catalytic function of the nucleotides. The activation by NAD(H) is competitively inhibited by cyclic 3',5'-AMP (3). Geynet et al. (4) examined isomerase activity in beef adrenal microsome fragments obtained by treatment with 0.5 - 2.0 M $CaCl_2$ (or other divalent cation salts) at pH 7, and suggested that the enzyme existed as a micellar aggregate containing a divalent cation-phospholipid-protein complex. Some support for this view was afforded by the demonstration that beef adrenal microsomal isomerase activity was destroyed by treatment with phospholipase A, and could be restored by addition of total microsomal lipids (5).

An interesting activation phenomenon was observed by Hamilton et al. (6, and references cited), who observed that the conversion of pregnenolone to progesterone by rat adrenal microsomes was stimulated by rat, bovine, and human serum albumins (and possibly by lactalbumin), but was not stimulated by ovalbumin. The stimulatory effect was on the 3β-hydroxysteroid dehydrogenase activity; the isomerase reaction was not affected (6).

This paper describes and examines the mechanism of the profound activational effect of various mammalian albumins on the isomerase activity of bovine adrenal microsomes.

MATERIALS AND METHODS

Materials. Δ^5-Androstene-3,17-dione and Δ^5-pregnene-3,20-dione were synthesized (7). Solutions of crystalline bovine plasma albumin (Armour) and serum albumins from other species (U.S. Biochemicals) were neutralized with NaOH. All albumins were assumed to have the mol. wt. of 68,000, although in fact minor differences in mol. wt. exist (8). Conalbumin (mol. wt. 89,000) was from Schwarz/Mann; and chicken ovalbumin (mol. wt. 45,000) and whole bovine serum were from Pentex. Anti-bovine albumin was a rabbit IgG fraction (Cappel Laboratories) which contained 4.2 mg of antibody protein and 16.4 mg of total protein per ml. Trypsin (twice crystallized) was obtained from Worthington. Triton X-100 was from Sigma. Concentrations of Triton X-100 were determined spectrophotometrically at 275 nm (2.153 ml $mg^{-1}cm^{-1}$), and based on an average mol. wt. of 636 (9).

Preparation of Microsomes. Two types of tissue were used in these studies: whole frozen calf adrenals and fresh bovine adrenal cortex (defatted and demedullated). All operations were conducted at 0-4°. Tissues were homogenized in a Waring Blendor with 3 volumes of 50 mM Tris HCl, 250 mM sucrose, 5 mM $MgCl_2$, 25 mM KCl (pH adjusted with HCl to 7.0 at 4°). Successive centrifugations at 600 x g, 6,000 x g, 20,000 x g, and finally at 105,000 x g for 2 hours, yielded the microsomal pellets. Calf adrenal microsomes were

suspended in the above medium at a protein concentration of 17.9 mg/ml. Beef adrenal cortical microsomes were washed once by centrifugation and resuspended in the medium at a protein concentration of 33 mg/ml. These preparations were frozen rapidly and stored at -80°. Aliquots were thawed and diluted 10-fold with distilled water just prior to use. Protein concentrations were determined by the method of Lowry et al. (10).

Enzyme Assays. The standard spectrophotometric assay system for Δ^5-3-ketosteroid isomerase activity contained 100 mM potassium phosphate at pH 7.0, and either 68 μM Δ^5-androstene-3,17-dione and 0.67% (v/v) methanol, or 10 μM Δ^5-pregnene-3,20-dione and 3.3% methanol. The microsomal suspension was added to initiate the reaction. The initial velocity of the formation of the product, Δ^4-androstene-3,17-dione (a_M = 16,300 $M^{-1}cm^{-1}$) or Δ^4-pregnene-3,20-dione (a_M = 17,000 $M^{-1}cm^{-1}$) was measured at 248 nm at 25° against a blank containing all components except the steroid. Corrections were made, when necessary, for nonenzymatic isomerization rates.

RESULTS AND DISCUSSION

Properties of the Steroid Isomerase; Effect of Serum Albumins. Microsomal preparations retained full isomerase activity for at least 1 month at -80°, and diluted aliquots lost no activity in 10 hours at 0°. Initial velocity of steroid isomerization was proportional to enzyme concentration both in the presence and absence of BSA. At pH 7.0, the rate of isomerization of

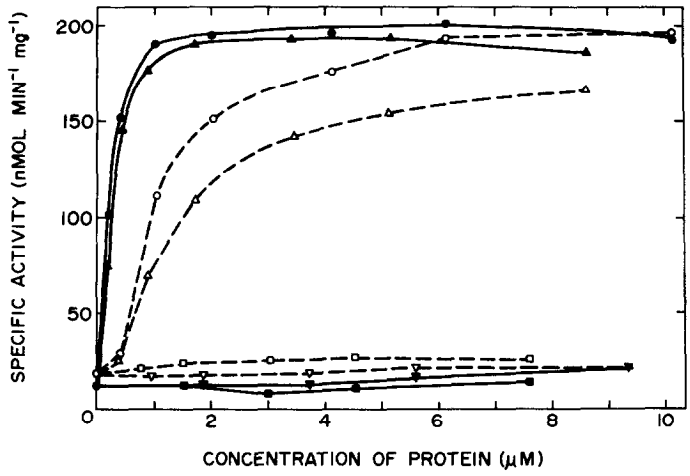

Fig. 1. Velocity of isomerization of Δ^5-androstene-3,17-dione (open symbols) and Δ^5-pregnene-3,20-dione (solid symbols) by calf adrenal microsomes, as a function of the concentrations of BSA (o and ●), human serum albumin (Δ and ▲), conalbumin (□ and ■), and chicken ovalbumin (▽ and ▼). These proteins had been dialyzed against 0.1 M potassium phosphate at pH 7.0. The assay systems contained 0.1 M potassium phosphate, pH 7.0, and either 65 μM Δ^5-androstene-3,17-dione, 0.67% methanol, and 11.9 μg of microsomal protein per ml, or 10 μM Δ^5-pregnene-3,20-dione, 3.3% methanol, and 3.0 μg of microsomal protein per ml.

Δ^5-androstene-3,17-dione by calf adrenal microsomes was increased 10-fold by 6 μM BSA, and that of Δ^5-pregnene-3,20-dione was enhanced 20-fold by 1-2 μM BSA (Fig. 1). Human serum albumin produced similar increases in these activities, whereas two unrelated proteins, ovalbumin and conalbumin, were virtually ineffective. Serum albumins of ten other species enhanced several-fold the rate of isomerization of Δ^5-androstene-3,17-dione by beef adrenal cortex microsomes (Table I).

Effect of pH. Figure 2A shows the effect of BSA on the pH-activity profile of the isomerization of Δ^5-androstene-3,17-dione by beef adrenal cortex mic-

TABLE I. Effect of Albumins on the Isomerization of Δ^5-Androstene-3, 17-dione by Beef Adrenal Cortex Microsomes

Addition to assay system[a]	Specific activity (nmol $min^{-1} mg^{-1}$ of microsomal protein)
None	119
Chicken ovalbumin	89
Conalbumin	116
Serum albumins:	
Rat	391
Human	394
Guinea Pig	461
Bovine[b]	487
Horse	498
Sheep	508
Goat	508
Rabbit[b]	528
Rabbit	537
Pig	545
Dog	556
Chicken	569

[a]The standard assay system was supplemented with the specified proteins at a concentration of 5 μM.

[b]Crystalline serum albumins. Others were Cohn Fraction V.

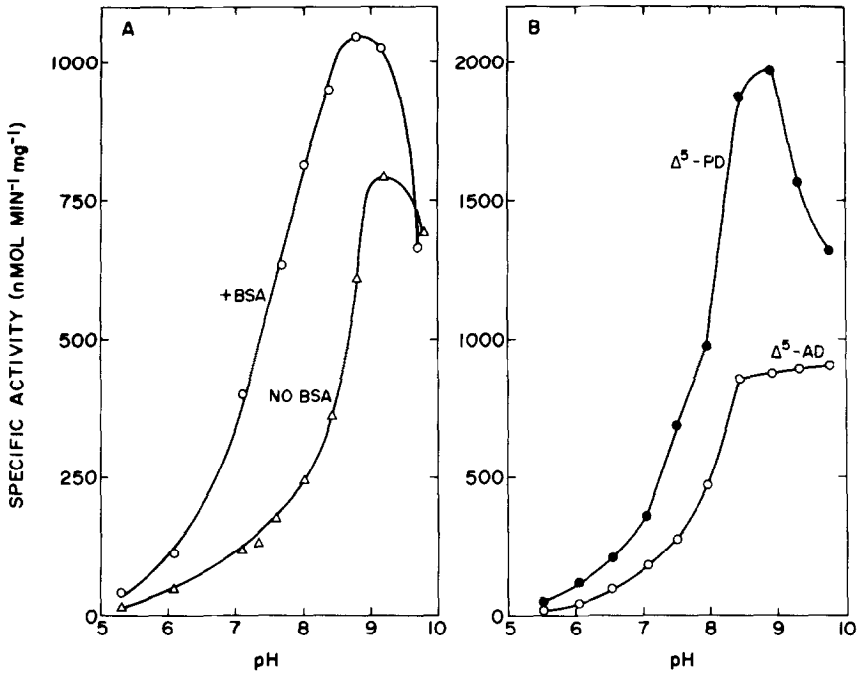

Fig. 2A. Velocity of isomerization of Δ^5-androstene-3,17-dione by beef adrenal cortex microsomes as a function of pH, in the absence of BSA (Δ) and in the presence of 5 μM BSA (o). Assays were conducted in Tris-potassium phosphate buffers at 25°, with 58 μM Δ^5-androstene-3,17-dione.
Fig. 2B. Velocity of isomerization Δ^5-androstene-3,17-dione (o) and Δ^5-pregnene-3,20-dione (•) by calf adrenal microsomes as a function of pH. Assays were conducted in Tris-potassium phosphate buffers at 25°, with 2.5 μM BSA, and either 60 μM Δ^5-androstene-3,17-dione or 5.3 μM Δ^5-pregnene-3,20-dione.

rosomes. As has been observed previously (11), isomerase activity increased with increasing pH. Maximal levels were observed at about pH 8.5-9 in the presence of BSA, and at a slightly higher pH in its absence. The activation by BSA is not due primarily to a shift in the pH optimum of the isomerase (Fig. 2A). The pH dependence of the isomerization of Δ^5-pregnene-3,20-dione by calf adrenal microsomes in the presence of BSA resembles that of Δ^5-androstene-3,17-dione (Fig. 2B). Some instability of these enzymatic activities in the assay systems was apparent at pH values above 8.5. In the absence of specific information as to the pH of the microenvironment of the adrenal isomerase in vivo, most of our studies have been performed at pH 7, at which pH the enzyme activities, while not optimal, exhibited marked stability.

TABLE II. Effect of BSA on the Kinetic Parameters for Isomerization of Δ^5-Androstene-3,17-dione

Source of microsomes	BSA (μM)	K_m ± S.E. (μM)	V_{max} ± S.E. (nmol $min^{-1} mg^{-1}$)
Calf adrenal	0	54.6 ± 0.4	27.8 ± 0.1
	0.51	41.9 ± 5.1	63 ± 4
	0.82	40.0 ± 2.2	134 ± 4
	1.23	38.0 ± 2.7	189 ± 7
	2.05	41.9 ± 4.4	235 ± 13
	4.11	39.5 ± 3.7	269 ± 12
	6.16	35.8 ± 5.7	268 ± 20
	8.22	38.5 ± 0.4	296 ± 1
	10.27	39.2 ± 1.7	311 ± 6
Beef adrenal cortex	0	61.5 ± 11.7	215 ± 21
	0.10	25.7 ± 3.9	268 ± 13
	0.25	19.4 ± 5.9	378 ± 31
	0.50	21.4 ± 5.6	452 ± 34
	5.00	23.0 ± 2.0	655 ± 18

Kinetics of the Activation by BSA. The effects of BSA concentration on the kinetic constants for the isomerization of Δ^5-androstene-3,17-dione are shown in Table II. Even the lowest concentrations of BSA tested (0.1 - 0.5 μM) elicited a decrease in K_m (to 40-70% of the values observed in the absence of albumin), as well as an increase in V_{max}. Higher concentrations of BSA did not result in further changes in K_m, but the V_{max} values rose progressively as the BSA concentrations were increased. The small effects of BSA on the K_m values for Δ^5-androstene-3,17-dione might possibly result from enhanced solubility of the steroid in the aqueous system. However, such effects are not likely to account for the large increases in V_{max}

which are primarily responsible for the activation by BSA. The concentration of BSA required for half-maximal activation of isomerization of Δ^5-androstene-3,17-dione by calf adrenal microsomes was 1.57 - 1.62 μM and did not appear to vary with the concentration of the steroid over the range of 15.5 - 62 μM. Thus, the reaction rate is unlikely to be controlled by the concentration of a steroid-BSA complex.

Inhibition by Triton X-100. In the absence of BSA, the nonionic detergent Triton X-100 (3 - 186 μM in the assay system) did not affect the isomerase activity of calf adrenal microsomes with Δ^5-androstene-3,17-dione as substrate. However, in the same system, these levels of Triton X-100 efficiently prevented the activation produced by 1 μM BSA (Fig. 3). As little as 11 μM Triton X-100 reduced the activational effect of BSA by one-half. This effect of Triton X-100 was not observed when either BSA or calf adrenal microsomes were first incubated with relatively high levels of the detergent (372 μM) and then diluted into the assay system. Thus the effect of Triton X-100 is related to its final concentration in the assay system.

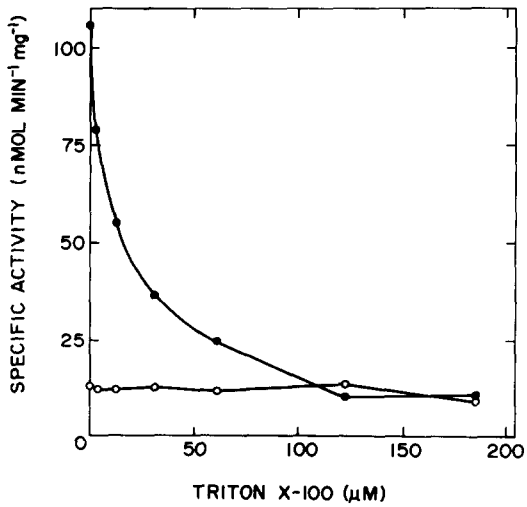

Fig. 3. Effect of Triton X-100 on the BSA-mediated activation of the isomerization of Δ^5-androstene-3,17-dione by calf adrenal microsomes. The assay systems contained 0.1 M potassium phosphate at pH 7.0, 68 μM Δ^5-androstene-3,17-dione, 0.67% methanol, 6.0 μg of microsomal protein per ml, and either 1 μM BSA (●) or no BSA (o).

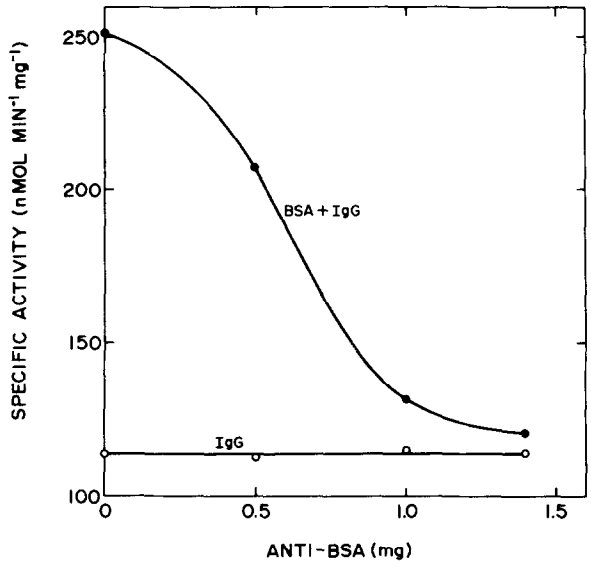

Fig. 4. Immunoprecipitation of activator by rabbit anti-BSA IgG. A series of solutions containing 0.1 mg of BSA and either 0, 0.5, 1.0, or 1.4 mg of antibody protein in 0.4 ml of 0.15 M NaCl-0.1 M potassium phosphate at pH 7.1, were incubated at 4° for 16 hours. After centrifugation, equal portions of each of the supernatant fractions (sufficient to yield a maximal concentration of 0.2 μM BSA in the assay system) were tested for their ability to enhance the rate of isomerization of Δ^5-androstene-3,17-dione by beef adrenal cortex microsomes (•). A parallel series of incubations which contained antibody (and no BSA) served as a control (o).

Examination of the Nature of the Activator. The question of whether the activation of the microsomal steroid isomerase was due to the BSA itself, or to a contaminant in these preparations, was considered. The activator could not be removed from BSA by extraction of an aqueous solution of the protein with chloroform, by dialysis, or by gel filtration on Sephadex G-150. Heating a solution of BSA at 100° for 10 min resulted in 88% loss of activity, and incubation with trypsin (5%, w/w) at 25° for 26 hours resulted in progressive and finally total loss of the activator potency. Whole bovine serum, which also activated the microsomal steroid isomerase, was subjected to gel filtration on Sephadex G-25; the activator was eluted at the void volume of the column. Precipitation of BSA by specific antibody resulted in loss of activator potency from the supernatant fraction (Fig. 4), with essentially all of the activator

being removed at an antibody protein concentration of 14 mg per mg of BSA. We conclude that BSA is directly responsible for the activation of the microsomal steroid isomerase.

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