BETA GLUCURONIDASE-RICH CYTOPLASMIC PARTICLES IN ANDROGEN-STIMULATED MOUSE KIDNEY

A Cytobiochemical Study

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ABSTRACT

Androgens produced by stimulating mouse testis with gonadotropic hormones cause a rise in renal β-glucuronidase but not an increase in acid or alkaline phosphatase. All subcellular components increase in β-glucuronidase activity, with a relatively greater increment in particulate enzyme as compared with that free in the cytoplasm (non-sedimentable). A small percentage of recovered β-glucuronidase, acid phosphatase, and alkaline phosphatase is found in material which rises to the surface during centrifugation in sucrose media (fraction I). The specific activity of β-glucuronidase and acid phosphatase in this fraction is normally quite high with respect to the homogenate, while that of alkaline phosphatase is not. On the other hand, the fraction I material from androgen-stimulated mice exhibits a further increase in specific activity with respect to β-glucuronidase and not acid phosphatase. It thus appears that there is an independence in the behavior of individual enzymes in response to physiologic stimuli in spite of obvious morphologic proximity.

INTRODUCTION

The ability of testosterone to elevate mouse renal β-glucuronidase concentration and enhance its excretion into the urine has been amply confirmed. Moreover, exogenous androgenic compounds (6, 7), and hormones (5) stimulating testicular interstitial cells, have all exhibited potency in this regard. The elevation of enzyme activity appears to be remarkable in the sense that it is far greater in extent than one would expect from the over-all protein anabolic effect and renotrophic action of these hormones. Moreover, a number of other enzymes in the same tissues are not increased in activity following the administration of these materials. Also, other tissues such as liver show no change in activity of β-glucuronidase after androgen administration. In this report, several experiences with cellular fractionation of androgen-stimulated mouse renal tissue are recorded. A species of cytoplasmic particle has been identified as a site of high enzyme activity, and this fact serves to clarify in some respects the nature of the kidney response of β-glucuronidase to androgens.

METHODS

Male mice of the AJAX strain aged 1.5 to 4 months were sacrificed by cervical fracture. Their kidneys were removed, decapsulated, and weighed. A 10 per cent homogenate with cold 1.75 M sucrose in 0.34 M NaCl was made with a ground glass homogenizer, and 2.0 cc of this homogenate was transferred to each of several celluloid centrifuge tubes. A solution of cold 1.08 M (37 per cent) sucrose

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in 0.34 M NaCl was carefully layered over the homogenates to fill the tubes; these were spun at 36,000 RPM (120,000 *g*) in a Spinco high speed refrigerated centrifuge with the no. 40 fixed angle rotor head. Fig. 1 shows the location of phases in the tube before and after centrifugation. Fractions were quantitatively harvested with a long needle and syringe, pooled, and assayed for β-glucuronidase (1), acid phosphatase (2), alkaline phosphatase (3), and protein (4). A qualitative assay was done for succinic dehydrogenase by a modification of the Thunberg method (9). All aliquots taken for assay were diluted 1:10 with distilled water before assay, since our findings concurred with those of other investigators who have shown that high concentrations of sucrose are inhibitory to several enzyme systems, including β-glucuronidase (8). The use of Triton X-100 was omitted in this study, since in our hands it does not enhance β-glucuronidase activity in particulate preparations. Specific activity in the case of each enzyme is expressed as units per milligram of protein.

Androgen stimulation of mice was accomplished by the intraperitoneal injection of 10 International Units of Follutein (human chorionic gonadotrophin) on each of two consecutive days, the animals being sacrificed 48 hours after the second injection. Female mice were not studied.

In Table I, values are listed for protein and the total and specific enzyme activities of homogenate plus fractions prepared from mouse kidney of control and androgen-stimulated animals. The specific activities of fractions I to V in this experiment appear also in Fig. 1.

RESULTS

There is a 6- to 7-fold elevation in total β-glucuronidase in androgen-treated animals as compared with controls (Table I). Total acid and alkaline phosphatase activities are not significantly increased by androgen stimulation.

The top or "floating" fraction I in control and stimulated mice consistently contains a low percentage of the total renal β-glucuronidase, but its specific activity is on the order of 2 to 20 times that of the homogenate, and higher than that of other fractions. Acid phosphatase, also low in absolute activity in this fraction, has a specific activity significantly greater than that of the homogenate. While both β-glucuronidase and acid phosphatase showed increases in specific activity in fraction I obtained from control and stimulated animals, only β-glucuronidase increased in total activity. Alkaline phosphatase, on the other hand, exhibits neither an increase in total activity in stimulated animals nor an increase in specific activity in fraction I. Succinic dehydrogenase activity is absent or immeasurably small in fraction I.

Fraction II, representing the bulk of sucrose solution overlying the homogenate, shows a con-

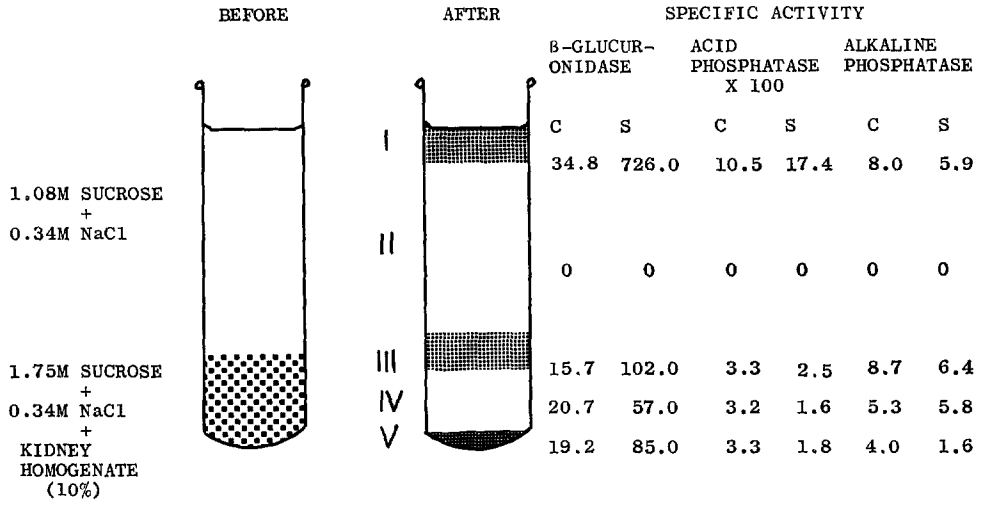

FIGURE 1

Appearance of phases in centrifuge tube before and after centrifugation. and their specific enzyme activities. I to V, fractions; C, control kidney; S, androgen-stimulated kidney.

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TABLE I

Comparison of Assay Data in the Homogenate and Several Fractions of Stimulated and Control Mouse Renal Tissue

	Fractions (% recovery in parentheses)					Homogenate	Total recovery
	I	II	III	IV	V		
CONTROL ANIMALS							
Protein (mgm)	10.3 (1.62%)	0	250.0 (39.3%)	108.0 (17.0%)	153.0 (24.1%)	635.7	82.0%
β-Glucuronidase (Fishman units)	359.0 (3.22%)	0	3,910.0 (35.1%)	2,240.0 (21.0%)	2,940.0 (26.4%)	11,116.0	85.7%
β-Glucuronidase (specific activity)	34.8	—	15.7	20.7	19.2	17.5	—
Acid phosphatase (King-Armstrong units)	1.08 (10.7%)	0	8.26 (81.8%)	3.45 (34.1%)	5.01 (49.6%)	10.11	176.2%
Acid phosphatase (specific activity × 100)	10.50	—	3.30	3.20	3.27	1.59	—
Alkaline phosphatase (Bodansky units)	82.0 (2.19%)	0	2,160.0 (57.8%)	577.0 (15.4%)	604.0 (17.1%)	3,740.0	92.5%
Alkaline phosphatase (specific activity)	7.96	—	8.65	5.33	3.95	5.86	—
Succinic dehydrogenase (scale 0 to 4)	0	0	4	1	0	4	—
ANDROGEN-STIMULATED ANIMALS							
Protein (mgm)	3.25 (0.383%)	0.10 (0.012%)	407.0 (48.0%)	138.0 (16.3%)	253.0 (29.8%)	849.0	94.5%
β-Glucuronidase (Fishman units)	2,360.0 (3.27%)	0	41,500.0 (57.4%)	7,830.0 (10.8%)	21,400.0 (29.6%)	72,282.0	101.1%
β-Glucuronidase (specific activity)	726.0	—	102.0	57.0	85.0	85.3	—
Acid phosphatase (King-Armstrong units)	0.565 (4.38%)	0	10.1 (78.2%)	2.25 (17.4%)	4.62 (35.8%)	12.9	135.8%
Acid phosphatase (specific activity ×100)	17.40	—	2.49	1.630	1.825	1.52	—
Alkaline phosphatase (Bodansky units)	19.0 (0.452%)	0	2,600.0 (62.0%)	800.0 (19.0%)	409.0 (9.73%)	4,200.0	91.2%
Alkaline phosphatase (specific activity)	5.85	—	6.39	5.80	1.62	4.95	—
Succinic dehydrogenase (scale 0 to 4)	0	0	4	1	0	4	—

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sistent *absence* of all the enzymes assayed, and protein is present only in negligible amounts. Fraction III, accounting for one-third to one-half of the protein recovered, is composed of mitochondria (as evidenced by strong succinic dehydrogenase activity) and probably microsomes, although specific assays to characterize the latter are not included. Fraction IV represents the clear liquor remaining at the site of the original homogenate, and is particle-free by microscopic examination. The pellet, fraction V, by microscopic examination, is composed of nuclei and incompletely ruptured tissue cells.

In an attempt to increase the yield of β-glucuronidase in fraction I, experiments (Fig. 2) were carried out in which the concentration of the overlying sucrose solution was altered in stepwise fashion. Non-stimulated mouse kidney was used. There appears to be a direct relationship between concentration of overlying sucrose and the yield of β-glucuronidase in fraction I. However, this is accompanied by a fall in specific activity of fraction I enzyme, probably due to contamination with protein from fraction III. If the overlying sucrose concentration exceeds 1.33 M (47 per cent), fraction III is dispersed in clumps throughout it, and clean separation of fractions I and III is not achieved. Variation of concentration of overlying sucrose does not significantly alter fractions IV and V. Fraction IV β-glucuronidase exhibits a consistent average of 200 units per hour (specific activity 3 to 8), and fraction V β-glucuronidase, an average of 210 units per hour (specific activity 16 to 26) regardless of the concentration of sucrose layered above the homogenate.

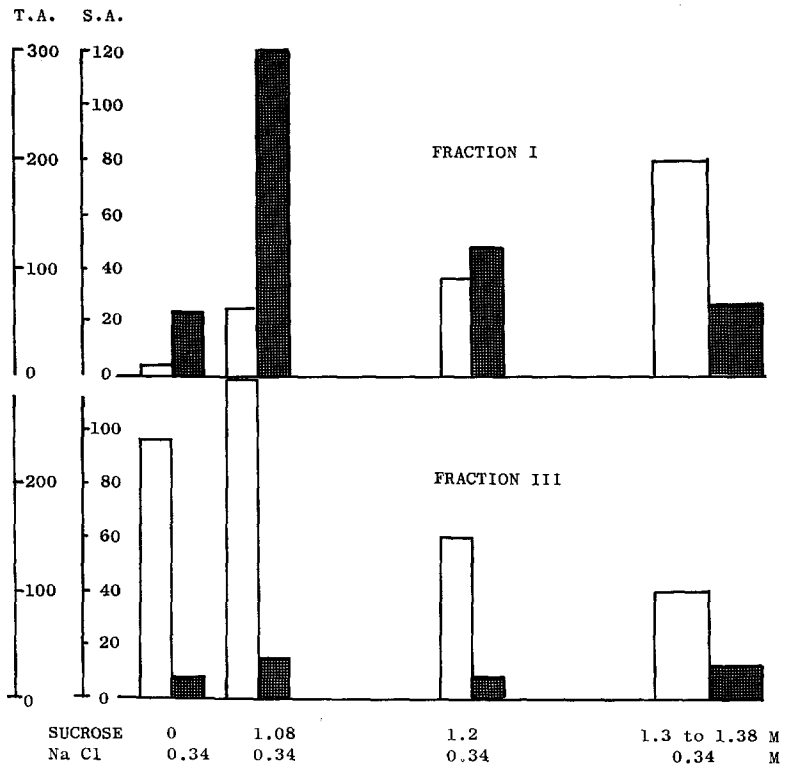

FIGURE 2

Comparison of total and specific activity of β-glucuronidase in fractions I and III with variation in concentration of overlying sucrose solution. Non-stimulated renal tissue. The white bars signify total activity (T.A.) and the hatched bars represent specific activity (S.A.)

DISCUSSION

Cellular fractionation of a number of tissues from various animals has shown that β-glucuronidase,

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for the most part, is associated with a cellular organelle which tends to sediment in the mitochondrial fractions with high speed centrifugation. De Duve (10) has succeeded in isolating the lysosome from these fractions, and has shown that it contains several hydrolytic enzymes, among which is β-glucuronidase. Experiences in our laboratory in fractionating mouse renal tissue have generally been directed to locating the specific cellular organelle, if any, which participates in the striking elevation of total renal β-glucuronidase when animals are treated with androgens or testicular stimulating hormones. To date, our experience has been that all particulate and non-sedimentable fractions participate in the β-glucuronidase response; minor differences among the particulate fractions have been inconstant and statistically insignificant. In general, however, particulate enzyme seems to increase more than "soluble phase" enzyme in absolute terms; this excess is distributed more or less equally among the sedimentable fractions. The fractionation summarized in Table I shows this differential elevation of particulate enzyme in stimulated mice, the rise in the free or "soluble phase" enzyme (fraction IV) being of lower magnitude.

The relatively high specific activity of β-glucuronidase and acid phosphatase in particulate matter which "floats" during centrifugation is of interest. The system employed in this study is the end result of attempts at fractionation of renal tissue, utilizing an overlying sucrose density gradient such as that of Kuff and Dalton (11), which suggests the possibility that fraction I might be Golgi membrane material. The relative concentrations of β-glucuronidase and acid phosphatase in this material are reminiscent of lysosomes; however, the integrity of these particles clearly is not adversely affected by the concentrated sucrose solutions employed, nor is Triton detergent required for their disruption. Also, this fraction accounts for only 3 per cent of the total cellular enzyme. The possibility cannot be excluded that this material is found in only one of the many cell types encountered in the kidney. That it differs in density from the other particles to be found in fraction III is shown by its complete separation from fraction III by an intervening sucrose solution lacking protein and the enzymes assayed in these experiments. It should also be noted that liver phagosomes are associated with lipid layers (12) which "float" in sucrose solutions upon centrifugation.

It is unlikely that all cellular fractions participate in the β-glucuronidase response although each does become richer in β-glucuronidase. A more likely explanation is that the over-all rise is a result of extensive intracellular migrations of the particulate enzyme under various conditions; such migrations of particles have already been shown to occur by the techniques of histochemistry. Straus (13) has recently found that the brush border of rat renal tubules bears spherical particles (phagosomes) which stain intensely for horseradish peroxidase after the parenteral infusion of this foreign protein. Peroxidase-rich granules also appear in the central areas of the cell. Fishman (14) has histochemical evidence that androgenic stimulation of renal tissue in the mouse produces similar bodies along the tubular lumen which are rich in β-glucuronidase. Moreover, work by Pettengill and Fishman (15) has shown that the elevation in renal β-glucuronidase by androgen stimulation represents an actual synthesis of enzyme protein, as opposed to the activation of "latent" previously synthesized enzyme. Since evidence is abundant that the microsomes account for a great percentage of protein synthesis in the intact cell, one can surmise that appearance of recently synthesized β-glucuronidase in the several cell fractions represents a migration of enzyme from the microsomal areas after its production. This enzyme may end up in cytoplasmic particles of varying dimensions and mass, one species of which has been separated physically and characterized enzymically in the experiments reported in this paper.

Finally, although several enzymes may be found within subcellular particles which have identical specific gravity and mass, it appears that an individual enzyme may still respond uniquely and specifically to a physiologic stimulus.

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BIBLIOGRAPHY

1. FISHMAN, W. H., SPRINGER, B., and BRUNETTI, R., *J. Biol. Chem.*, 1948, **173,** 449.
2. STOLBACH, L. L., NISSELBAUM, J. S., and FISHMAN, W. H., *Am. J. Clin. Path.*, 1958, **29,** 379.
3. SHINOWARA, G. Y., JONES, L. M., and REINHART, H. L., *J. Biol. Chem.*, 1942, **142,** 921.
4. LOWRY, O. H., ROSEBROUGH, W. J., FARR, A. L., and RANDELL, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
5. FISHMAN, W. H., BENJAMIN, G. S., and GREEN, S., *J. Biol. Chem.*, 1956, **222,** 351.
6. FISHMAN, W. H., ARTENSTEIN, M., and GREEN, S., *Endocrinology*, 1955, **57,** 646.
7. FISHMAN, W. H., *in* Mechanism of Action of Steroid Hormones, (C. A. Villee and L. L. Engel, editors), Oxford, Pergamon Press, 1961, 157.
8. CONCHIE, J., MAY, A. J., and LEVVY, G. A., *Biochem. J.*, 1961, **79,** 324.
9. BONNER, W.D., Methods in Enzymology, (S. P. Colowick and N. O. Kaplan, editors), New York, Academic Press, Inc., 1955, **1,** 722.
10. DE DUVE, C., *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press Co., 1959, 128.
11. KUFF, E. L., and DALTON, A. J., *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press Co., 1959, 114.
12. STRAUS, W., *J. Biophysic, and Biochem. Cytol.* 1959, **5,** 193.
13. STRAUS, W., *J. Histochem. and Cytochem.*, 1962, **10** 672.
14. FISHMAN, W. H., unpublished observations.
15. PETTENGILL, O. S., and FISHMAN, W. H., *Exp. Cell Research*, 1962, **28,** 248.

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