Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 633–634. Pergamon Press. Printed in Great Britain

SHORT COMMUNICATION

INABILITY TO ISOLATE THE 3β-HYDROXYSTEROID DEHYDROGENASE FROM BOVINE OVARIES USING A PROCEDURE WHICH ISOLATES THE ADRENAL ENZYME

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(*Received* 22 *September* 1975)

SUMMARY

A simple method involving Triton extraction followed by ECTEOLA cellulose chromatography which resulted in the purification of the 3β-hydroxysteroid dehydrogenase [EC 1.1.1.51] present in bovine adrenal microsomes to a single homogeneous protein [1], did not similarly purify the enzyme present in ovarian microsomes. Our criteria of purity involved the use of SDS gel electrophoresis with gradient gels in contrast to the constant composition gels used for the adrenal enzyme.

The 3β-hydroxysteroid dehydrogenase [EC 1.1.1.51] present in bovine adrenal microsomes was purified to a single homogeneous protein by a simple procedure involving extraction with Triton X-100 followed by ECTEOLA cellulose chromatography [1]. This report described the first case of the complete purification of the extremely important steroid metabolizing enzyme which is required for the production of all physiologically active steroid hormones, and which has been suggested to be involved in the control of steroid metabolism in placenta [2].

However, certain aspects of the study were disturbing. The degree of purification of the enzyme was extremely low; the dehydrogenase was not separated from the 5-ene isomerase and the enzyme was not eluted from the ECTEOLA cellulose but had passed through the column unretarded. As we have been studying the 3β-hydroxysteroid dehydrogenase in bovine ovaries [3], we investigated this purification procedure using the ovarian microsomal enzyme in order to compare the properties of the enzymes in the two tissues.

Whole bovine ovaries without regard for the stage of the estrus cycle were homogenized in 0.068 M sodium phosphate buffer, pH 7.0, containing 10 mM β-mercaptoethanol and 0.25 M sucrose. Cell debris and nuclei were removed by centrifugation at 500 *g* for 15 min and the supernatant fluid was spun consecutively at 3500 *g* for 15 min, 10,000 *g* for 15 min and 105,000 *g* for 90 min, the sedimented particles being removed at each step. The microsomal fraction (particles which sedimented at 105,000 *g* for 90 min) was washed once by resuspension and recentrifugation in isotonic KCl. The microsomes were treated with Triton X-100 and the extract chromatographed on a column of ECTEOLA cellulose as described [1]. The Triton X-100 extract was analyzed by SDS gel electrophoresis on slab gels conctructed with a gradient of polyacrylamide [4]. The enzymic activity was assayed using radioactive steroid substrates (Gibb and Hagerman, submitted for publication). Protein concentrations were determined as described [5].

The degree of purification of the 3β-hydroxysteroid dehydrogenase from bovine ovaries with both dehydroepiandrosterone and pregnenolone as substrates (Table 1) was similar to that obtained for the adrenal enzyme [1]. The enzyme also had similar chromatographic properties, in that it passed through the ECTEOLA cellulose column

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Table 1. Purification of the 3β-hydroxysteroid dehydrogenase activity from bovine ovaries

Fraction	Substrate*	Specific activity†	Yield (%)	Purification (-fold)
500 *g*	D	0.189	100	
supernatant	P	0.220	100	
Microsomes	D	1.17	65	6.6
	P	1.24	61	5.6
KCl washed	D	1.32	65	7
microsomes	P	1.63	61	7.4
1% Triton X-100	D	1.74	53	9
extract	P	1.53	35	7
ECTEOLA-	D	2.90	19	15
cellulose extract	P	1.96	11	9

* D—dehydroepiandrosterone; P—pregnenolone.
† nmol × min^{-1} × mg^{-1} protein.

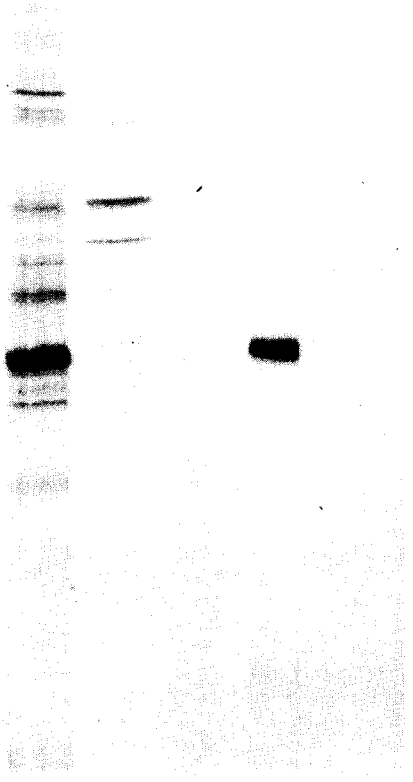

Fig. 1. SDS polyacrylamide gel of Triton X-100 extract of ovarian microsomes prior to ECTEOLA-cellulose chromatography (left), after ECTEOLA-cellulose chromatography (center) and albumin (right).

unretarded. However, in contrast to the adrenal enzyme, ECTEOLA cellulose chromatography did not result in the purification of the ovarian enzyme to a single homogeneous protein (Fig. 1).

The study on the adrenal enzyme indicated that a single 3β-hydroxysteroid dehydrogenase metabolizes pregnenolone and dehydrocpiandrosterone. The present findings would allow the possibility that there are multiple enzymes and corresponds with our results on the Triton X-100 extract of ovarian microsomes whereby the stereospecificity of hydride transfer from dehydroepiandrosterone and pregnenolone has shown that there are at *least two* 3β-hydroxysteroid dehydrogenases [6].

Although the 5-ene-isomerase is considered to be a separate enzyme from the 3β-hydroxysteroid dehydrogenase [7], the study of the adrenal did not result in separation of the activities. The investigators did not assay their purified protein for any other enzymic activity known to be present in adrenal microsomes e.g. the 17α-hydroxylase or C-21 lyase, nor did they examine whether cytochrome P-450 or 420 was present in the preparation.

There may be several reasons for the discrepancy between our results with the ovarian enzyme and those obtained with the adrenal enzyme [1]. The most obvious possibility is that different tissues were used in the two studies and the observed difference is a property of the organ or cell types, although in both instances the 3β-hydroxysteroid dehydrogenase activity passed through the ECTEOLA cellulose column unretarded and the degree of purification achieved was about the same. It is also possible that we found multiple proteins in the ECTEOLA cellulose eluate because we used a gel electrophoresis system with a gradient of polyacrylamide and hence a higher resolving power, in contrast to the constant composition gels used in the earlier work.

Despite the difference in the source of the enzymes the relatively low degree of purification and similar column chromatographic properties of both the adrenal and ovarian enzymes and other aspects of the adrenal study mentioned suggests that a more rigorous examination of the purity of the adrenal enzyme is warranted prior to concluding that it has been purified to homogeneity.

Acknowledgements—This work was supported by NICHD Research Grant No. HD-05085. S.S.S. was the recipient of an NIH postdoctoral fellowship and W.G. was the recipient of a postdoctoral fellowship from the Population Council (N.Y.).

REFERENCES

1. Ford H. C. and Engel L. L.: *J. biol. Chem.* **249** (1974) 1363–1368.
2. Townsley J. D.: *Acta endocr., Copenh.* **79** (1975) 740–748.
3. Gibb W. and Hagerman D. D.: *Fedn. Proc.* **33** (1974) 1574.
4. Shapiro S. S., Phiromsawat S. and Hagerman D. D.: *Biochem. biophys. Res. Commun.* **62** (1975) 376–381.
5. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. S.: *J. biol. Chem.* **193,** (1951) 265–275.
6. Gibb W. and Hagerman D. D.: *Can. Fedn. biol. Soc.* **18,** (1975) 95.
7. Cheatum S. G. and Warren J. C.: Biochem. biophys. Acta **122** (1966) 1–13.