Pak. j. sci. ind. res., vol. 33, no. 12, December, 1990

IDENTIFICATION OF ANDROGEN-DEPENDENT EPIDIDYMAL PROTEINS IN THE GOAT *(CAPRA HIRCUS)*

AZRA KHANUM, M.Z. HAIDER, WASIM AHMAD* AND S.M.S. NAQVI**
Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

(Received September 16, 1989; revised January 1, 1991)

The androgen dependence of specific epididymal proteins have been studied in the adult goat *(Capra hircus)*. At least six distinct proteins in the pre-albumin area have been shown to be synthesized in the goat epididymis under androgen stimulus. In the androgen deprived animals, four pre-albumin proteins disappeared and there was marked reduction in the colour intensity of the other two protein bands. Testosterone treatment for 14 days, however, restored the normal pattern of pre-albumin proteins. Electrofocusing of epididimal cytosol from intact, castrated and testosterone treated castrated goats further confirmed the presence of six androgen-dependent proteins having pI's 4.2-5.9 and molecular weight between 16,000-48,000 daltons.

Key words: Epididymis, Proteins, Androgen.

Introduction

Mammalian epididymis is known to provide an essential environment for spermatozoa to undergo maturational changes [1-7]. Various studies have shown that epididymal secretions are mainly dependent on androgens and epididymal plasma has an unusually high concentration of certain constituents such as proteins, carnitine, glycerylphosphorylcholine and several hydrolytic enzymes [4,6,8-15]. Available evidences further indicate that androgen-dependent epididymal secretory proteins interact with maturating spermatozoa of lizard [15], rat [11,16], rabbit and hamster [17] and coat the sperm surface [18-20]. These proteins have been generally characterized as glycoproteins [20-22], acidic proteins [23,24] and forward motility proteins [25]. Recent studies further mentioned that specific proteins secreted in the epididymis have an important physiological role in the development of motility and fertilizing capacity of spermatozoa after they leave the testis [14,26-28]. Therefore, in view of the potential significance of specific epididymal proteins on sperm maturation, an attempt has been made to identify the proteins which are regulated by testosterone in goat epididymis, since it is an economically important species.

Materials and Methods

Chemicals and animals. All chemicals used in the present study were of analytical grade. Standard protein markers for the calibration of gel electrophoresis were obtained from Pharmacia Fine Chamical (Uppsala, Sweden). Ampholine PAG-plates pH 3.5-9.5 used for isoelectric focusing were the product of LKB-produkter (Bromma, Sweden).

The adult goats *(Capra hircus)* used in this study weighed from 17-18 Kg. Three animals were bilaterally castrated under Rompun anaesthesia (Bayer Leverkusen, Germany) and left for 45 days. At the end of this period, one epididymis was removed from the castrated goats. These animals subsequently received 15 mg testosterone propionate (Schering AG, Berlin, Germany) daily for 14 days. Twenty four hours following the last injection, the remaining epididymis was removed from each animal. Epididymides were also obtained from three intact adult animals which served as the controls. Upon removal, the epididymides were weighed, freed of adhering tissue and immersed in ice-cold 50 mM Tris buffer (pH 7.4) containing 1.5 mM EDTA.

Preparation of cytosol. Epididymal cytosol fraction was prepared as described by Haider *et al.* [7]. Aliquots of the cytosol were stored at 50° until used for analysis. The protein content in epididymal cytosol was determined by the method of Lowry *et al*. [29], using bovine serum albumin (BSA) as standard.

ELECTROPHORESIS:

Non-denaturing gels. Polyacrylamide gel electrophoresis was performed according to the method described by Raymond and Weintraub [30]. The gels were fixed in 10% sulphosalicylic acid, stained with 1% coomassie brilliant blue R-250 in 25% methanol, 10% acetic acid, and destained in 25% methanol, 10% acetic acid.

Denaturing gels. Electrophoresis under denaturing conditions was carried out in 10% polyacrylamide gels in tris-glycine buffer system, as described by Weber and Osborn [31]. The control, castrated and testosterone treated epididymal cytosol samples were run in parallel with standard protein markers: phosphorylase b (mol. wt., 95,000), BSA (mol. wt., 68,000), ovalbumin (mol. wt., 43,000), carbonic anhydrase (mol. wt. 31,200), *Soyabean trypsin* inhibitor (mol. wt., 20,100) and α lactalbumin (mol. wt. 14,000). The molecular

* Clinical Research Centre, Middlesex, U. K.
** Biology Department, M. E. T, University Ankara, Turkey.

weights of specific epididymal proteins were calculated according to the method of Weber *et al.* [31].

Electrofocusing. Electrofocusing in polyacrylamide gels was performed using ampholine plates (pH range: 3.5-9.5), as described by Haider *et al.* [7]. The stained gels were dried using a hot air blower and preserved in plastic sheets. The gels were scanned by using E-C model densitometer equipped with a kipp-zonen integrating recorder at a chart speed of 20 mm/min. The isoelectric points (pI's) for the specific protein bands were estimated by plotting a pH gradient (pH vs 1 mm of gel segments).

Results and Discussion

The data related to epididymal weight and total protein concentration in the cytosol fractions obtained from intact, castrated and castrated animals receiving testosterone propionate for 14 days, are given in Table 1. The castrated animals showed a decrease in the epididymal weight as compared to the intact ones at the end of the castraction period: whereas there is marked increase in the weight of epididymis following testosterone treatment to castrated animals. Our results are in agreement with that reported by Brooks [5] in the rat epididymis, where castration is followed by a decline in the weight of the epididymis and other accessory organs and an increase occurs in the weight with testosterone treatment. Similarly, Haider *et al.* [7] have reported an increase in the epididymal weight in castrated rhesus monkeys following testosterone treatment for six days.

The available evidence further suggests that there is maximal increase in the content of epididymal protein 24 hrs following administration of a single dose of testosterone to castrated rats [4]. Brooks [12] has reported that androgen causes upto a 2-fold increase in protein synthesis per unit weight in the rat epididymal tissue. An increase in protein content has also been shown in castrated rhesus monkeys 48 hrs following administration of testosterone [7]. Similarly, our study has shown an increase in the concentration of epididymal proteins in castrated goats after a two week testosterone propionate treatment period (Table 1).

TABLE 1. EPIDIDYMAL WEIGHT AND THE TOTAL PROTEIN CONCENTRATION IN CYTOSOL FRACTION.

Animals (adult)	No. of animals	Epididymal weight (g)	Protein concentration in epididymal cytosol (mg /g tissue)
Intact	3	4.626±0.62*	19.423±0.60
Castrated	3	3.503±0.33	16.011±1.52
Castrated and testosterone treated	3	4.648±0.53	24.765±0.56

*Mean ± S.D.

In this study, castration of animals for 45 days and subsequent administration of testosterone propionate to castrated animals was used as a model for monitoring the androgen-dependent proteins of the adult goat epididymis. Electrophoresis (non-denaturing) of cytosol fractions prepared from intact and testosterone treated castrated animals revealed the presence of six distinct protein components: I,II,III,IV,V and VI with Rf values 0.59, 0.63, 0.70, 0.76, 0.84 and 0.92 respectively. In the epididymal cytosol of castrated animals four of these bands: I,III, IV and V were absent in the electrophorograms and the concentration of bands II and VI was markedly reduced (Fig. 1). These results of electrophoresis suggest that synthesis of these protein components are under the testosterone influence. Previous studies, which characterize androgen-dependent epididymal proteins in the rat, have demonstrated the presence of four major bands in the pre-albumin area [11]. Brooks and Higgins [19] have also studied the androgen dependence of proteins associated with luminal fluid in the rat epididymis. These authors have also been able to demonstrate four major pre-albumin bands (B-E) in the luminal fluid obtained from the cauda. Haider *et al.* [7,32] have reported the synthesis of the least four distinct proteins in the immature and five in the adult monkey epididymis by testosterone treatment. However, Jones *et al.* [9] have described six polypeptides in the rat epididymis, whose synthesis has been shown to be hormonally regulated, by measuring the incorporation of ^{35}S methionine. Similarly, Echeverria *et al.* [6] have reported six androgen-dependent protein bands in hamster epididymis by using labelled amino acids.

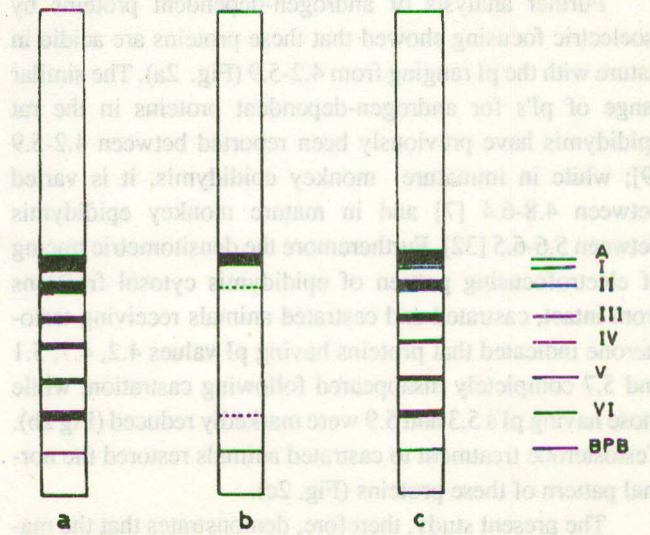

Fig. 1. Electrophoresis under non-denaturing conditions of epididymal cytosol fraction from (a) normal goats: (b) untreated castrated goats: (c) castrated goats treated with testosterone for 14 days.

Analysis of cytosol proteins obtained from epididymides on intact, castrated and testosterone treated castrated animals by electrophoresis under denaturing conditions indicated a pattern which is essentially similar to that obtained under the non-denaturing conditions. In this electrophoresis system, relative mobilities of pre-albumin bands (I-VI) were found to be as followed: I, 0.24, II, 0.246; III, 0.396; IV, 0.58; V, 0.63; VI, 0.83; the molecular weights of these proteins were: I, 48,500; II; 48,000 ; III; 39,000; IV, 27,500; V, 25,000; VI, 16,000. Therefore, SDS-polyacrylamide gel electrophoresis of cytosol prepared from intact, castrated and testosterone treated castrated animals revealed that the specific epididymal proteins lack disulphide bridges and most probably do not have subunit structure. Lacke of subunit structure in the androgen-dependent proteins has also been reported in the laboratory rodents [6,19] and primates [7,32]. The molecular weights of these proteins ranges between 16,000-48,000. These values are different from those reported in rhesus monkey, hamster, rat and lizard [7,11,28,32], suggesting that these proteins are apparently not identical with those present in lizard, rat hamster and rhesus monkey. Depeiges *et al.* [28] have provided the evidence for the presence of at least six epididymal proteins in lizard during the month of April with molecular weights 19,000-94,000. Similarly, Jones *et al.* [9] have described same number of epididymal proteins in rat with molecular weights ranged from 18,500-80,000. Furthermore, Echeverria *et al.* [6] have also showed six androgen sensitive proteins in hamster with quite different molecular weights 41,000-61,000, whereas Haider *et al.* have demonstrated four epididymal proteins having molecular weight 47,000-66,000 in the immature rhesus monkey epididymis.

Further analysis of androgen-dependent proteins by isoelectric focusing showed that these proteins are acidic in nature with the pI ranging from 4.2-5.9 (Fig. 2a). The similar range of pI's for androgen-dependent proteins in the rat epididymis have previously been reported between 4.2-5.9 [9]; while in immature monkey epididymis, it is varied between 4.8-6.4 [7] and in mature monkey epididymis between 5.6-6.5 [32]. Furtheremore the densitometric tracing of electrofocusing pattren of epididymis cytosol fractions from intact, castrated and castrated animals receiving testosterone indicated that proteins having pI values 4.2, 4.7, 5.1 and 5.7 completely disappeared following castration, while those having pI's 5.3 and 5.9 were markedly reduced (Fig 2b). Testosterone treatment to castrated animals restored the normal pattern of these proteins (Fig. 2c).

The present study, therefore, demonstrates that the mature goat epididymis like other animals is dependent on the contineous supply of androgen for its functional activity. At least six epididymal protein species exhibit androgen depend-

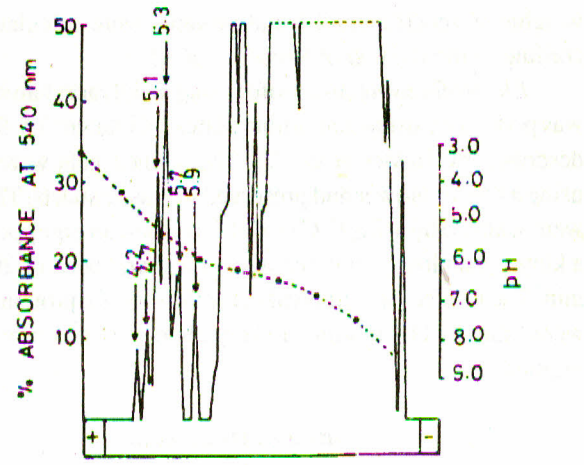

Fig. 2a. Densitometric scan of epididymal cytosol proteins after electrofocusing in normal animals. Absorbence (——), pH gradient (- - - -).

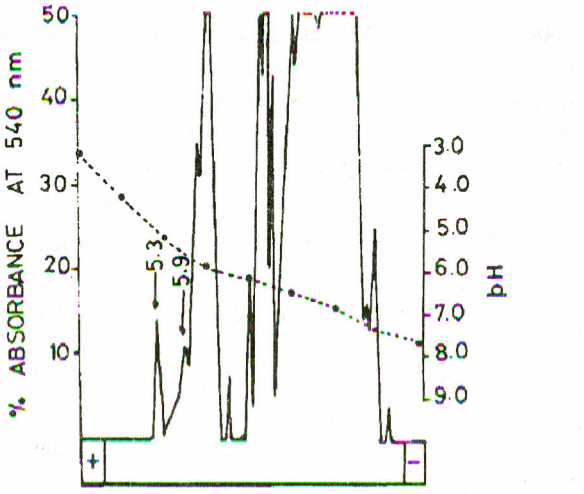

Fig. 2b. Densitometric scan of epididymal cytosol proteins after eclectrofocusing in untreated castrated animals. Absorbence (——), pH gradient (- - - -).

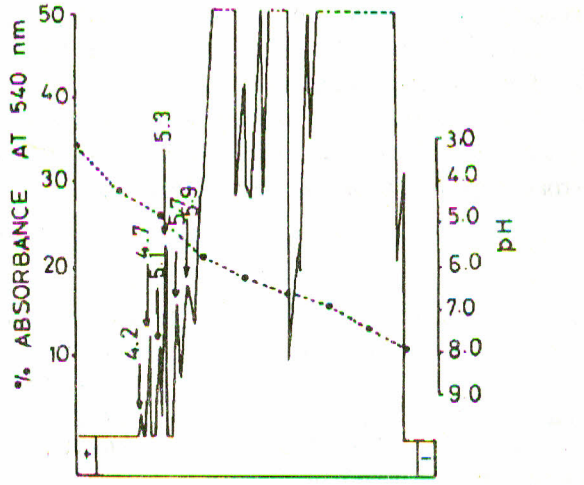

Fig. 2c. Densitometric scan of epididymal cytosol proteins after electrofocusing in castrated goat treated with testosterone for 14 days. Absorbence (——), pH gradient (- - - -).

ency. These proteins are acidic in nature with pI's ranging from 4.2-5.9 and having molecular weights between 16,000-48,000. Further studies may be undertaken to elucidate the physiological role played by the specific e pididymal androgen-depen-dent proteins, especially their function in association with spermatozoa. The data obtained here would provide the basis for further investigation of the role of epididymis in sperm maturation and thus on the reproductive ability in this very important farm animal in Pakistan.

References

1. T. Mann, *The Biochemistry of Semen and of the Whale Reproductive Tract,* (Mathuen, London, 1964), 2nd ed., pp. 37.
2. J.A. Blaquier, M.S. Cameo and M.H. Burgos, Endocrinology, **90**, 839 (1972).
3. M.C. Orgebin-Crist, B.J. Danzo and J. Davies, *Hand Book of Physiology, Endocrinology* (1975), Vol. 5th, pp. 319-338 .
4. M.R.N. Parasad and M. Rajalakshmi, Gen. Comp. Endocrinol., **28**, 530 (1976).
5. D.E. Brooks, J. Endocrinol., **82**, 305 (1979).
6. F.M.G. Echeverria, P.S. Cuasnicu and J.A. Blaquier, J. Reprod. Fert., **84**, 1 (1982).
7. M.Z. Haider, M.H. Qazi, A. Khanum and M. Arslan, Amer. J. Primatol., **4** , 73 (1983).
8. R. Jones, Comp. Biochem. Physiol., **61B**, 365 (1978).
9. R. Jones, C.R. Brown, I.V.G. Karoly and M.G. Parker, Biochem. J., **188**, 667 (1980).
10. R.K. Rastogi, J. Steroid Biochem., **11**, 101 (1979).
11. M.S. Comeo and J.A. Blaquier, J. Endocrinol., **69**, 47 (1976).
12. D.E. Brooks, Mol. Cell Endocrinol., **29**, 225 (1983).
13. W.R. Kelce and V.K. Ganjam, Steroids, **52**, 217 (1988).
14. M.E. Newcomer and D.E. Ong, J. Biol. Chem., **265**, 12876 (1990).
15. Y. Courty, F. Morel and J.P. Duafaure, J. Reprod. Fertil., **81**, 443 (1987).
16. A.C. Kohane, M.S. Cameo, L. Pineire, J.C. Garberi and J.A. Blaquier, Biol. Reprod., **23**, 181 (1980).
17. H.D.M. Moore, Biol. Reprod., **22**, 705 (1980).
18. J.C. Garberi, A.C. Kohane, M.S. Cameo and J.A. Blaquier, Mol. Cell, Endocrinol., **13**, 73 (1979).
19. D.E. Brooks and S.J. Higgins, J. Reprod. Fertil., **59** , 363 (1980).
20. D.E. Brooks, A.R. Means, E.J. Wright, S.P. Singh and K.K. Tiver, Eur. J. Biochem., **161**, 13 (1986).
21. J.M. Bedford, Nature, **100**, 1178 (1963).
22. F. Dacheux and J.L. Dacheux, Cell Tissue Res. , **255**, 371 (1989).
23. O.A. Lea, P. Petrusz and F.S. French, Inter. J. Androl. (Supp), **2**, 592 (1978).
24. N.J. Charest, P. Petrusz, P. Ordronneau and D.R. Joseph, Endocrinology, **125**, 942 (1989).
25. H. Brandt, T.S. Acott, D.J. Johnson and D.D.Hoskins, Biol. Reprod., **19**, 830 (1978).
26. K.H. Scheit, M. Kemme, G. Aumuller and J. Seitz, Biosci. Rep., **8**, 589 (1988).
27. M.L. Lopez, P. Grez, I. Gribbel and E. Bustos Obregon, J. Submicrosc. Cyto., Pathol., **21**, 103 (1989).
28. A. Depeiges, A. Force and J.P. Dufaure, Comp. Biochem. Physiol., **86**, 233 (1987).
29. O.H. Lowry, N.J. Rosenbrough, A.L. Farr and A.G. Randall, J. Biol. Chem., **193**, 265 (1951).
30. S. Raymond and J. Weintraub, J. Science, **130**, 711 (1959).
31. K. Weber and M. Osborn, J. Biol. Chem., **244**, 4406 (1969).
32. M. Arslan, M.Z. Haider and M.H. Qazi, Arch. Androl., **16**, 67 (1986).