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Analysis of Calcium Binding Proteins of Mouse Epididymal Spermatozoa

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INTRODUCTION

Mammalian testicular sperm is unable to fertilize the oocyte and it must undergo "maturation" during the epididymal transit. Ejaculated sperm, however, still needs a final maturation process in the female genital tract, collectively described as sperm capacitation, to obtain the ability to react acrosome reaction (AR). In AR in a Ca^{2+}-dependent manner, penetrate the investments of oocyte, and eventually fuse with oolemma (reviemed in Yanagimachi, 1994).

It has been reported that extensive changes in physiological and biochemical properties of sperm occur during sperm matuation in epididymis (reviewed in Cooper, 1986). As a result, sperm surface antigens involved in the recognition and binding to egg investments got matured and sperm became able to react physiological acrosome reaction. in meet with egg investments. Undoubtedly, acrosome reaction, a Ca^{2+}-dependent ecocytotic event is essential for fertilization and regulated by unique cellular and environmental factors associated with either gametes or the reproductive tracts (reviewed in Yanagimachi, 1994). Ca^{2+} is major signal in acrosome reaction of spermatozoa. Interactions between sperm surface receptor(s) and extracellular ligands generate signal propagated intracellurly and subsequent signal cascade leads to Ca^{2+} influx. In spite of the independent studies on the several proteins responsible for intracellular calcium signaling during the past decade, protein data base for calcium binding protein of spermatozoa has not been established.

So far, the analysis of calcium binding proteins was solely dependent on the isotopic methods. Recently non isotopic detection of calcium binging proteins by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2D SDS-PAGE) was developed (Isamu and Hitoshi, 1997). Present study aimed to examine the changes in calcium binding proteins of mouse epididymal sperm during epididymal transit and AR by 2D SDS-PAGE.

MATERIALS & METHODS

Epididymis were removed from 3 months-old male mice (ICR strain). Blood was cleared from tissues by blotting to filter paper and the organ was dissected into three parts descending the length of epididymis, Caput, corpus, and caudal region of epididymis were placed in the modified Tyrode solution, and tissues were squeezed with forceps to release their luminal contents. Sperm suspension was collected after 10 min incubation at RT and cleared from cellular debris by repeated filtration through the lens paper. After the sperm concentration was adjusted to 2 X 10^6 sperm/ml with fresh medium, 1 ml of aliqouts of cauda epididymal sperm suspension was placed in the 4-well dish and preincubated in 5% CO2 and 95 % air at 37℃. After 120 min, Ca^{2+}-ionophore A23187 (10 uM in 0.1% DMSO) was added to sperm suspension and incubated for further 60 min to induce AR.

After centrifugation at 1,000 g for 10 min sperm pellet was mixed with 20 volumes of extraction buffer (20mM Tris-HCl, pH 7.5, 1% triton X-100, proteinase inhibitor cocktail) and incubated for 1 hr at 4℃. Clear supernatant was collected after 10,000 g for 30 min and mixed with SDS sample buffer (Laemmli, 1970) and subjected to 2D SDS-PAGE. Two-dimensional SDS-PAGE was conducted according to Isamu and Hitoshi (1997). First dimensional SDS-PAGE was the same as reported in Laemmli (1970) but 2mM EDTA was added to resolving gel mix before polymerization. After the 1st PAGE, gel was sliced into strip of 1.5mm in width. After the incubation of the slice in equilibrium buffer (0.125M Tris-HCl, pH 6.8, 20% glycerol, β-mercaptoethanol, 0.001%

bromophenol blue) for 10 min, gel slice was conducted to 2nd dimensional SDS-PAGE. Stacking gel was made as same as 1st dimension, but 2mM $CaCl_2$ was added to resolving gel mix before polymerization. To verify the mobility shift by protein-calcium interaction, gel slice made from 1st PAGE was resolved in 2nd PAGE gel containing 2mM EDTA or 2mM $ZnCl_2$, and migration pattern of each protein spots was compared after siver staining of the gel (Merril et al., 1981).

RESULTS & DISCUSSION

Changes in CBP profiles as well as the qualitatitive changes was apparent in epididymal sperm during their transit through the epididymis. It suggested that the ability of epididymal sperm to undergo AR are closely related with changes in CBPs during epididymal transit. Quantity of calmodulin, a major CBP of the spermatozoa which has MW 20kDa was reduced in acrosome reacted sperm. Other CBPs with varying size of MW were also decreased after the AR. It suggested presence of plasma membrane-bound or acrosomal vesicle-associated CBPs. Many spots of proteins from 2D SDS-PAGE actually shifted in the presence of Ca^{2+}. Since each deviated composed of a single polypeptide, 2D SDS-PAGE is a very convinient and powerful technique for detecting Ca^{2+}-binding proteins in crude extract of spermatozoa.

SUMMARY

The changes in calcium binding protein (CBP) of mouse epididymal sperm during their post-testicular differentiation were analyzed by two-dimensional SDS-PAGE. According to epididymal maturation, capacitation and acrosome reaction of spermatozoa, both quantitative and qualitative changes of CBPs in the epididymal sperm was detected. It suggested that the development of fertilizing ability of epididymal sperm was closely related to the changes in the CBPs profiles of sperm during epidiymal transit.

Key words : calcium binging protein, sperm, mouse

REFERENCES

Cooper TG (1986) The Epididymis, Sperm Maturation and Fertilization, Springer-Verlag, New York, pp 1-5.

Isamu K and Hitoshi F (1997) *Analytical Biochemistry* 249: 252-255.

Kopf GS and Gerton GL (1991) Elements of Mammalian Fertrtilization vol 1 (Wassarman P.M., eds.). Boca Raton, Florida, pp 153-203.

Laemmli UK (1970) *Nature* 227: 680-685.

Merril CR, Goldman D, Sedman SA, and Ebert MH (1981) *Science* 211: 1437-1438.

Yanagimachi R (1994) The Physiology of Reproduction (Knobil, E. and J. Neill, eds.). Raven Press, New York, Vol 1, pp 189-317.