Genetic Polymorphism of Boar Seminal Plasma Proteins

Soichi TSUJI, Masatoshi ASAO, Hiroshi KUSUNOKI and Takao OISHI

Faculty of Agriculture, Kobe University, Nada-ku, Kobe-shi 657
* National Institute of Animal Industry, Tsukuba Norinkenyudanchi Ibaraki-ken 305

(Received September 7, 1992)

Abstract In two breeds of Chinese boar, the Meishan and the Jinhua, the major seminal plasma secretory proteins exhibit a very different and distinct electrophoretic mobility pattern. Multiple protein bands were observed in Jinhua, and a predominant band in Meishan breed, besides, the mobility of these bands was also different. After removing the sugar moieties from these proteins, both breeds exhibited two predominant bands on SDS polyacrylamide gel. These results suggest that the seminal plasma in boar consists of two major protein molecules of differing sugar moieties. Neuraminidase treated proteins revealed that the major difference was due to the attachment of different number of sialic acid residues to the sugar moieties. It was also shown that the seminal vesicular secretory proteins of Meishan boar contained lower sialic acid than that of the Jinhua. Experiments using hybrid and backcross generations between a Meishan sow and a Jinhua boar showed that the characteristics of the seminal plasma proteins of the Jinhua breed are inherited as a dominant trait.


Key words: boar seminal protein, genetic variation, isoelectric-focusing, Chinese breeds, sialic acid

Since the amount of semen is abundant in boar as well as in stallion, extensive studies have been conducted on seminal plasma components. Seminal plasma proteins have been isolated and characterized revealing their protein nature and hemagglutinating function. Most studies have been conducted to elucidate the effect of seminal plasma proteins on fertility. Surprisingly even the removal of the accessory sex glands allows full fertilizing capacity of the semen. Thus, the function of the seminal plasma proteins remains unresolved. Two Chinese breeds, Meishan and Jinhua, from mainland China have been introduced into the Kobe University Experimental Farm. Since no information is available about seminal plasma proteins of Chinese breeds here the seminal plasma of the Meishan and Jinhua boars were examined by utilizing ultrathin-layer polyacrylamide gel isoelectric focusing (UTLIEF) and revealed genetically controlled polymorphisms of its proteins.

Materials and Methods

Semen was collected from 3 boars of the Meishan breed, 2 boars of the Jinhua breed, 9 boars from a F1 litter between a Meishan sow and a Jinhua boar and 10 boars from a litter of a backcross between one of the F1 boars and a Meishan sow manually and filtered these through a double gauze to remove gel particles. Semen was centrifuged at 10,000 rpm for 10 min. to remove spermatozoa and the seminal
plasma was stored at -20°C until analysis. The prostate and the seminal vesicles were removed from a Meishan boar and a Jinhua boar immediately after slaughter, and the fluid of these organs was squeezed out of the ducts. In most of the experiments, seminal plasma and treated plasma were analyzed by UTIEF using Ampholine ranging from pH 3.5 to pH 10.0. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was also employed to analyze these samples. Sugar moiety was removed from seminal plasma proteins as well as sialyl residue by using neuraminidase of Vibrio cholerae type II. The content of sialic acid was determined by periodate-resorcinol reaction.

Results and Discussion

1. Polymorphism of boar seminal plasma proteins

The pattern of seminal plasma of the three Meishan boars clearly differed from those of the two Jinhua boars (Fig. 1). Seminal plasma of Jinhua boars had one major band, named number 3 and three minor bands, numbers 1, 2 and 4, while that of Meishan showed one major band, named number 1, accompanied by few bands of lower intensity (Nos. 2 and 4). In comparison between the major band of Meishan and that of Jinhua, the band of Jinhua (No. 3) represent a basic protein having higher isoelectric point and more acidic than that of Meishan (No. 1). LAVON and BOURSNELL also reported similar differences of seminal plasma proteins between individuals of European breeds which showed that most of their boars had the Jinhua type of proteins with four distinct bands, but they did not pursue their studies further.

Intensive studies have been conducted, which revealed that the major proteins are produced in the seminal vesicle. These reports suggested that the major proteins showing different electrophoretic mobility on UTIEF are also from the seminal vesicles. To confirm this, secretions of the seminal vesicle and the prostate collected from a Meishan and a Jinhua boar were analyzed by UTIEF. Fig. 2 clearly shows that the pattern of the seminal vesicle protein is almost identical to that of the seminal plasma proteins in both breeds. This therefore, suggests that the proteins are secreted from the seminal vesicle.

2. Removal of sugar moieties from seminal plasma proteins

The mobility of proteins in a polyacrylamide gel depends mainly on its size and charge. Modification of protein by sugar moieties also affects the mobility of the protein in a gel. In order to study the difference in electrophoretic mobility of seminal vesicular secretion proteins between the Meishan and the Jinhua breeds, seminal plasma proteins were deglycosylated by the method of EDGE et al. Usually deglycosylation makes proteins insoluble, therefore the SDS-PAGE method of LAEMMLI was employed to analyze the deglycosylated proteins. As shown in Fig. 3, untreated seminal plasma proteins showed different SDS-PAGE patterns between the Meishan and the Jinhua breeds. The difference is obvious at the anode portion on the gel (bottom part of Fig. 3).
UTLIEF, seminal plasma of Meishan had deeply stained one band and showed a simple pattern compared to that of Jinhua. However in SDS-PAGE, the patterns were more complicated with Meishan as well as Jinhua showing multiple bands. Deglycosylation had the pronounced effect of reducing the multiple bands to a few bands and the deglycosylated seminal plasma proteins of the Meishan boar like to those of the Jinhua boar consisted of two predominant bands. These results suggest that the differences in seminal vesicular proteins between the breeds on UTLIEF is dependent on the type of sugar moiety attached to the proteins. The mobility on SDS-PAGE depends on molecular size of protein, so, principally, there are two predominant protein molecules of different size in the seminal vesicle secretion. The modification of these two predominant proteins by sugar moieties results in multiple bands on SDS-PAGE and on UTLIEF. This also suggests that non-treated seminal plasma proteins actually consist of multiple protein molecules having a different number and/or different type of sugar moiety. Attachment of sialic acid residues to sugar moieties can explain the change of electrophoretic mobility of these proteins. Fig. 4 shows that neuraminidase treated Jinhua seminal plasma proteins exhibit the same pattern as those of untreated Meishan seminal plasma proteins. This result suggests that the number of sialyl residues being attached to sugar moieties on a protein domain causes the difference in electrophoretic mobility among breeds. Table 1 shows the sialic acid content of seminal plasma protein of two different Chinese breeds. In accordance with the results of neuraminidase treatment (Fig. 4) and the comparison of electrophoretic mobility between breeds (Fig. 1), Jinhua boars J1 and J2, showed a higher content of sialic acid in their seminal plasma proteins ranging from 12 µg to 17 µg/mg protein, while Meishan boars M1, M2 and M3...
Fig. 3. Effect of deglycosilation on seminal plasma proteins. Electrophoresis was carried out using sodium dodecylsulfate polyacrylamide gel of 12% gel. C, control; DG, deglycosilated; M, Meishan; J, Jinhua. Five mg of freeze dried protein was deglycosilated at 25°C for 1 hour in the presence of 0.33 ml of anisol and 0.66 ml of trifluoromethanesulfonate. Three μg of deglycosilated proteins and 8 μg of non-treated proteins was applied on the gel.

Fig. 4. Effect of neuraminidase treatment on electrophoretic mobility of seminal plasma proteins analyzed by ultrathin-layer polyacrylamide gel isoelectric focusing. C, control; DS, neuraminidase treated; M, Meishan; J, Jinhua. Three mg of protein was treated with 0.6 units of neuraminidase from Vibrio cholerae (Sigma Chem. Co. USA) for 48 hours at 37°C in the presence of 0.04 M CaCl₂. Three μg of proteins was applied on the gel.

Table 1. Content of sialic acid in seminal plasma protein. (μg sialic acid per mg of seminal plasma protein)

<table>
<thead>
<tr>
<th>Breed</th>
<th>Individual</th>
<th>Sialic acid content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meishan</td>
<td>1</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.6</td>
</tr>
<tr>
<td>Jinhua</td>
<td>1</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.8</td>
</tr>
</tbody>
</table>

showed a lower content of about 4 μg/mg of protein. Seminal plasma proteins were not fractionated into their components, so sialic acid residues may be contained not only in the major protein, but also in minor proteins. The sialic acid content presented here may not directly reflect sialic acid content of major seminal vesicular secretory proteins. Though the Meishan boars showed a lower content of sialic acid in their seminal plasma, the major seminal vesicular secretory proteins of the Meishan boar may not have sialic acid residues, because no changes of mobility were observed on the UTLIEF gel before and after treatment with neuraminidase.

3. Inheritance of the polymorphic variations of boar seminal plasma proteins

The variation in seminal vesicle secretory proteins between individuals and among breeds of boars may be shown to be genetically controlled. Seminal plasma proteins were collected from hybrid boars from a cross between a Meishan sow and a Jinhua boar and from backcross boars between a Meishan sow and one of the hybrid boars. The seminal plasma proteins were analyzed by UTLIEF. In this
Boar Seminal Plasma Protein Polymorphism

In back-cross breeding experiments (Fig. 5b) 4 out of 10 boars revealed the Jinhua type and the remaining 6 boars had the Meishan type pattern. The F1 hybrid and backcross experiments were conducted once and only one litter at each step was used. However, it could be concluded that the pattern of the Jinhua type is inherited as a simple Mendelian dominant trait.

From these results it is concluded that (1) the major seminal plasma secretory proteins studied consist of two predominant protein molecules of different sizes, (2) after modification with sugar moieties, following attachment of sialic acid residues, the proteins show multiple molecular forms with different electrophoretic mobilities on UTLIEF and on SDS-PAGE, (3) the different numbers of sialic acid residues attached to the protein molecule is inherited as a dominant trait and (4) there is a possibility that seminal vesicle secretory proteins in Meishan breed has no sialic acid residue due to the absence of sialyl transferase activity. Since in chicken there are several positive results showing that sialic acid of protein molecules is related to fertility\(^{10,12}\), the difference between sialic acid residues attached to seminal vesicular secretory proteins may have some effect on reproductive ability in pigs. Although this characteristic is not restricted to Meishan boars, further experiments will be necessary to reveal if modification of seminal vesicular proteins with sialic acids has any effects on the reproduction in boars.

Acknowledgements

We thank Dr. H. Tsuchida for helping to conduct this experiment and Mr. T. Sudo for his assistance in preparing this manuscript.
References


豚精漿蛋白質の遺伝的多型

辻 荘一・浅尾雅俊・楠 比呂志・大石孝雄*

神戸大学農学部、神戸市灘区 657

* 農林水産省畜産試験場、茨城県農林研究圏地，305

豚精漿の主な蛋白質は精巣腺由来である。中国豚の 2 品種梅山豚と金華豚の精漿蛋白質を超薄層等電点電気泳動法により分析したところ、両者の間に著しい違いが認められた。金華豚では複数のバンドが観察されるのに対して、梅山豚の精漿蛋白質では等電点の高いバンドが主として一本観察された。それらの蛋白質から糖鎖を除去すると SDS-ポリアクリルアミドゲル電気泳動では梅山豚、金華豚共に染色される一本のバンドとなった。このことから、豚精漿蛋白質は糖鎖の異なる 2 分子の蛋白質より成ることが示された。また、シアル酸の除去により、全豚豚型のバンドは梅山豚型となり、糖鎖へのシアル酸の付着数により、品種間の精漿蛋白質の差異が現われるものと考えられた。このことと関連して、梅山豚の精漿蛋白質のシアル酸の含有量は金華豚のそれに比べて低い値を示した。梅山豚と金華豚の交雑 F₁ ブタと、F₁ ブタと梅山豚との交配ブタの精漿蛋白質の分析の結果、金華豚の示す精漿蛋白質の多様性は梅山豚のそれに対して優性に遺伝すると推定される。

日畜会報, 64 (3) : 221-227, 1993