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ELECTROPHORETIC CHARACTERIZATION OF RAT BULBOURETHRAL GLYCOPROTEIN

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ABSTRACT

Electrophoresis of an extract from the bulbourethral glands under both denaturing and non-denaturing conditions and by two dimensional electrophoresis demonstrated that a substantial component of the extract is a glycoprotein. This bulbourethral glycoprotein apparently consists of a single polypeptide and has a molecular weight of 40 kiloDaltons. It focuses, in an isoelectric field, at pH 5.2.

INTRODUCTION

The bulbourethral glands are male sex accessory glands and are present in most mammalian orders (Price and Williams-Ashman, 1961). The paired rat bulbourethrals are mucous glands. Their cells' secretory products are deposited, by exocytosis, into acini that connect to a single duct. The duct empties directly into the urethra as a result of muscular contractions (Gueze and Slot, 1976, 1978). The bulbourethral secretion contributes to the general composition of preputial and seminal fluids.

Hart (1968), Hart and Greenstein (1968), Beil and Hart (1973), and Williams-Ashman et al. (1977) demonstrated that rat bulbourethral secretion accelerates the *in vitro* cross-linking of seminal vesicle secretory proteins by coagulating gland transglutaminase (EC 2.3.2.13). They suggested that this acceleration is an important factor in the *in vivo* formation of the vaginal plug. Williams-Ashman et al. (1977, 1980) and Williams-Ashman (1984) further concluded that the accelerating effect is due to the conformational modification of the basic protein substrate from the seminal vesicles by polyanionic macromolecules in the bulbourethral secretion. Hart and Greenstein (1968) reported that the rat bulbourethral secretion contains a sialic acid

rich glycoprotein and proposed that this is the active bulbourethral secretory factor that accelerates semen coagulation.

The experiments reported here were designed to identify some of the characteristics of bulbourethral glycoprotein(s) using electrophoretic procedures.

MATERIALS AND METHODS

Preparation of Bulbourethral Extract (BUE)

Male Sprague-Dawley retired breeders (purchased from Harlan Sprague Dawley Inc. Indianapolis, Ind.) were sacrificed using ether. Bulbourethral glands were removed, minced with scissors, and homogenized in 50 mM morphilinopropane sulfonic acid buffered at pH 7.4 and containing 1 mM ethylenediaminetetraacetic acid. Homogenates were then centrifuged at 100,000g for 60 minutes at 5°C in a Beckman model L5-50B ultracentrifuge. The pellet was discarded. Two volumes of acetone (−5°C) were added to the supernatant fraction while stirring over ice to precipitate dissolved proteins. This preparation was centrifuged at 15,000g for 15 minutes at 5°C in a Beckman model J-21B centrifuge. The pellet was washed three times with acetone (−5°C), air-dried overnight, and stored over desiccant at −10°C.

One Dimensional Gel Electrophoresis of BUE

This was accomplished with the use of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to a method described by Laemmli (1970). The gels contained 12% acrylamide and 0.18% N,N'-bis-acrylamide. No stacking gel was used.

Two Dimensional Gel Eectrophoresis of BUE

This procedure was conducted according to a method described by Roberts et al. (1984).

Gel Staining

The following staining procedure was employed in order to identify patterns of general protein separation. Tube gels and second dimension slabs were placed in isopropanol: acetic acid: water (10:10:80), overnight with one change, to fix the protein bands and to dissolve SDS from the gels. They were then placed in methanol: acetic acid: water (45:10:45) containing 0.25% Coomassie brilliant blue R for two hours. Finally, gels were treated with successive changes of methanol: acetic acid: water to dissolve any dye that was not bound to proteinaceous material.

To specifically stain for glycoproteins, the periodic acid Schiff's (PAS) procedure, described by Fairbanks et al. (1971), was used.

Molecular Weight Determination

The molecular weights of BUE polypeptides were determined by comparing their migration patterns with the migrations of known molecular weight standards purchased from Sigma Chemical Co.

RESULTS

Gel A in figure 1 illustrates the pattern of polypeptide banding obtained after one dimensional SDS-PAGE of whole BUE. This gel was stained with the general protein stain, Coomassie brilliant blue R. There are 10 intensely staining polypep-

tide bands ranging in molecular weight from approximately 120 to approximately 20 kiloDaltons (kD).

Gel B in figure 1 is a duplicate of gel A and was stained using the glycoprotein specific PAS procedure. Only the polypeptide band with the approximate molecular weight of 40 kD stained positively as a glycoprotein. To test whether the PAS staining procedure causes polypeptides to be removed from the gels, a PAS stained gel was subjected to the Coomassie staining regimen. All the polypeptide bands on the PAS stained gel were detectable after Coomassie staining, indicating that the pattern seen in gel B is the result of a specific interaction between stain and polypeptide rather than the result of elimination of other polypeptide bands by the PAS procedure.

To determine whether or not the bulbourethral glycoprotein is composed of smaller subunits that are bound together by difulfide bonds, BUE was dissolved in sample buffer containing 1% β-mercaptoethanol and electrophoresed. The migration pattern of the BUE glycoprotein remained identical to that seen in gel A, figure 1. Furthermore, in the absence of either of the denaturants, SDS and mercaptoethanol, the PAS positive polypeptide migrated just behind the ion front and near the anode. This is consistent with the pattern of a relatively low molecular weight, anionic macromolecule.

In order to study the electrofocusing characteristics of the BUE glycoprotein and to test further the possibility of more than one glycoprotein being present in the BUE, the extract was subjected to two dimensional PAGE. The resulting separation pattern is shown in figure 2. This gel was Coomassie stained. Several spots are detectable. The first dimension (left to right) is the separation using isoelectric focusing. Polypeptides ranging from slightly basic to highly acidic were observed. The second dimension (top to bottom) is a separation based on molecular weight. When a duplicate gel was stained by the PAS procedure, only the large polypeptide spot in the right center of the figure reacted positively. This spot electrofocuses at approximately pH 5.2, and its molecular weight is approximately 40 kD. This molecular weight value compares favorably with that for the PAS positive band on the one dimensional gels.

DISCUSSION

The evidence presented in this paper supports the conclusion that there is one glycoprotein with a molecular weight of approximately 40 kD in BUE. As judged by Coomassie staining, this polypeptide is the single most abundant component of the BUE.

The BUE glycoprotein's electrofocusing profile is apparently due to its carbohydrate content. Since the amino acid composition is not known, however, it is not possible to say to what extent acidic amino acids contribute. Hart (1971) conducted an amino acid analysis of a bulbourethral extract that was also prepared by acetone precipitation and reported high levels of both aspartic and glumatic acid residues. If these acidic amino acids are in the BUE glycoprotein they add to its anionic nature.

The presence in BUE of a large amount of anionic proteinaceous material is consistent with the contentions in the literature that a polyanionic factor from the bulbourethral gland is responsible for accelerating the post-ejaculatory formation of the vaginal plug. Conclusive confirmation of this proposal requires purification of the BUE glycoprotein and addition of the purified product to an *in vitro* reconstructed coagulation system such as described by Williams-Ashman et al. (1977).

The fact that mercaptoethanol did not change the BUE glycoprotein's migration pattern, indicates that it is not composed of smaller polypeptide subunits. Moreover, its migration pattern, in the absence of denaturants, is consistent with that of a relatively low molecular weight, anionic molecule, that is not a subunit of a larger molecule. It is likely that the BUE glycoprotein is secreted in the form that was detected in these studies.

Efforts to purify the BUE glycoprotein are currently underway in this laboratory. These studies should determine conclusively whether the BUE glycoprotein plays a role in the clotting acceleration and should lead to a better understanding of the acceleration mechanism.

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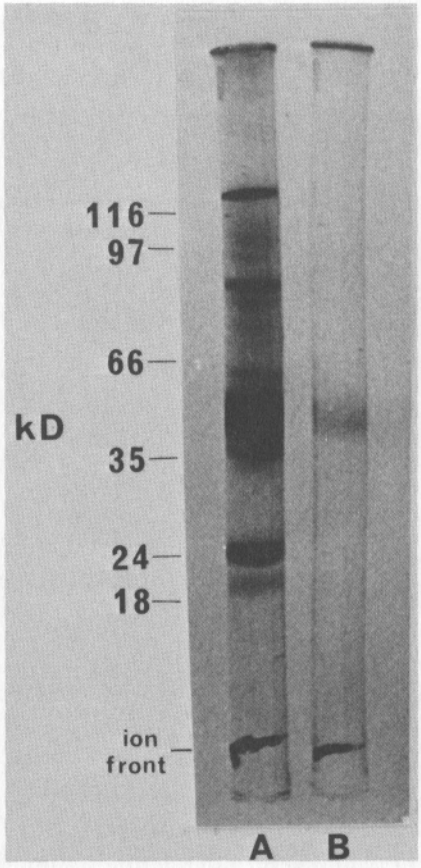

Fig. 1. One dimensional SDS-PAGE of BUE. A) Coomassie brilliant blue R stained gel B) PAS stained gel. The total amount of BUE electrophoresed on each gel was 50 μg.

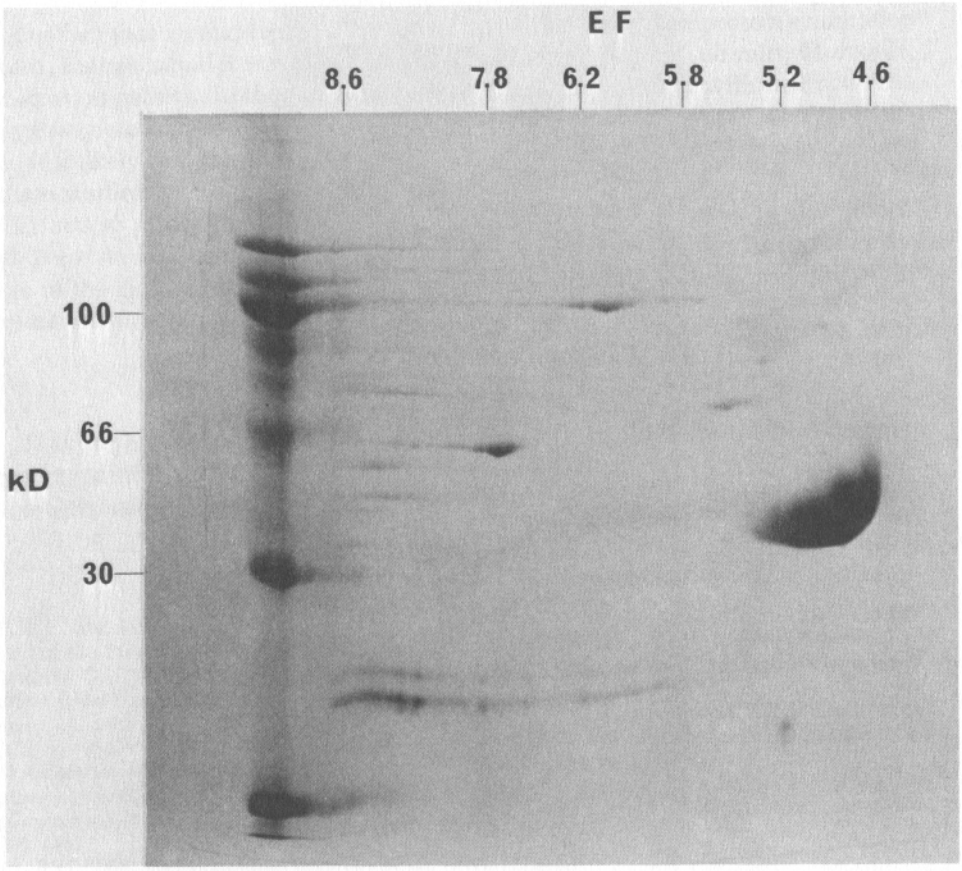

Fig. 2. Two dimensional PAGE of BUE. Isoelectric focus separation is from left to right and SDS-PAGE separation is from top to bottom. The lane of bands on the left side of the figure represents the migrations of molecular weight markers. The total BUE used was 50 μg.