A Disc-Electrophoresis Investigation of Certain Characteristics of Triton X-100 Solubilized Proteins from Male *Periplaneta americana*

Triton X-100 is frequently employed in the solubilization of pelletbound membrane proteins[1]. It is a non-ionic detergent which demonstrates a relatively low level of enzyme denaturation[2].

Disc-electrophoretic resolution[3] of Triton-solubilized proteins of *Periplaneta americana* antennae, from pellets previously extracted twice with 0.9% NaCl, yielded one major proteinaceous band. This fraction selectively bound the feeding inhibitor, ^{14}C-menadione[4]. SINGER and NORRIS[5] demonstrated the presence of a major proteinaceous band in the Triton X-100 solubilized portions of extracts from each of several different body regions of *P. americana*. In that study, SINGER and NORRIS found that the electrophoretic Rf value of this major Triton-solubilized band increased as the protein-sample load was increased. These observations suggested that this major band consisted of several aggregated proteinanceous components.

Studies were subsequently initiated to elucidate additional characteristics of this major electrophoreted band from the Triton extracts, and some of the findings are reported in this paper.

Materials and methods. Triton X-100 solubilized, pellet-bound, proteins were obtained from twice-saline-extracted pellets from extracts of antennae, heads, tarsi/tibiae, thoraces, abdomina and wings of *P. americana* males using 0.6, 1.0 or 5% Triton X-100 in 0.9% saline. Acrylamide gels of 7% concentration were normally employed, but different acrylamide concentrations between 3 and 14% also were compared to evaluate protein-sieving effects. Gels were formulated with, and without, Triton X-100 or SDS. Gels were buffered at pH 7.1 or 6.7 with Tris/HCl[3].

Continuous reservoir-buffer solutions included: a) *Tris*/glycine, pH 8.5 or 7.1; b) *Tris*/HCl, ph 7.1; c) Na phosphate, pH 8.5 or 7.1. Protein extracts in 20% sucrose were layered through the upper reservoir buffer on to the gels. Phenol Red (0.001%) functioned as the glycine-front marker in pH 8.5, while the glycine front was detectable as a yellow band during electrophoresis at pH 7.1. Electrophoreted gels were fix-stained in Amido Black-acetic acid. Other technique details have been published previously[5].

Results and discussion. Distinct and reproducible banding of Triton X-100 solubilized acidic proteins was accomplished only with *Tris*/glycine reservoir buffers. Electrophoresis of Triton-soluble extracts in acrylamide gels containing 0.6% Triton X-100 at pH 8.5 resolved one major proteinaceous fraction (Figure 1: antennae (a), heads (b), tarsi/tibiae (c), thoraces (d), wings (e) and abdomina (f)). This antennal protein resolved into one major band with an Rf value of 17 for 50 µg applied, 20 for 100 µg, 26 for 200 µg and 27 for 300 µg applied. Head protein resolved into a band with an Rf value of 9 for 110 µg applied, 15 for 220 µg and 23 for 440 µg. Tarsial/tibial protein produced a band with an Rf value of 11 for 118 µg applied and Rf 21 for 236 µg. Thoracic protein resolved into an Rf 11 band when 100 µg were applied and Rf 22 for 200 µg. Wing extracts resolved

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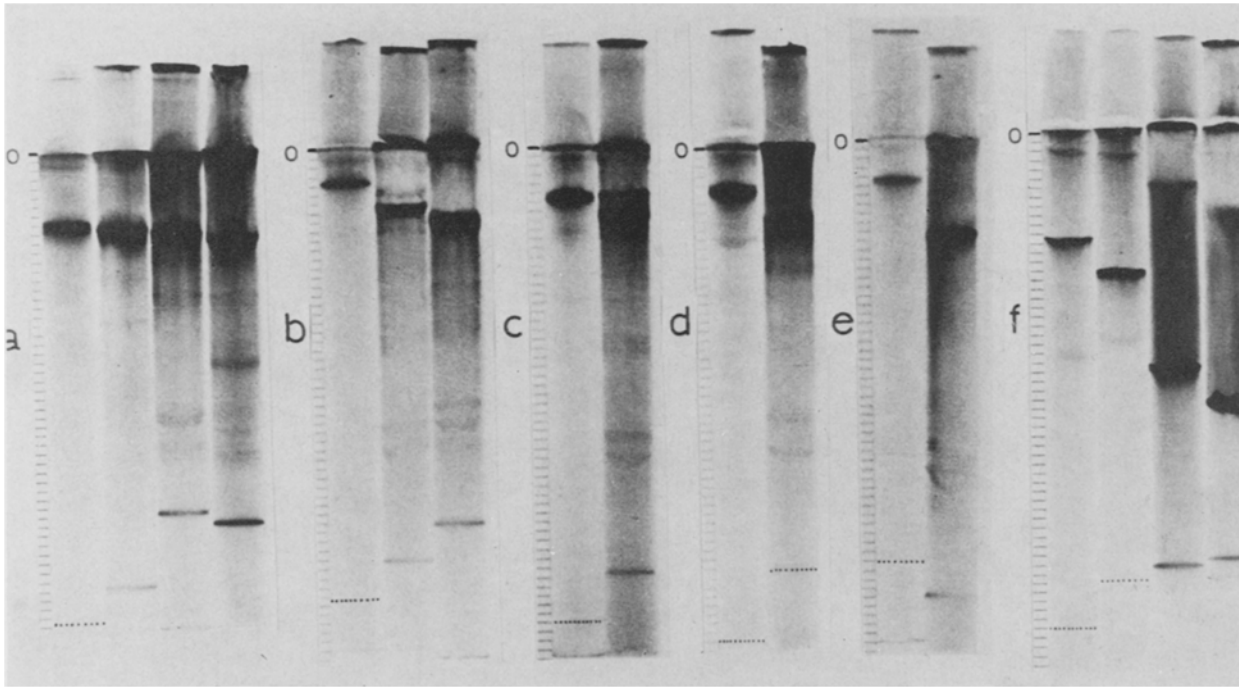

Fig. 1. Effects of protein concentration on the electrophoretic behavior of Triton-solubilized extracts in gels containing Triton X-100, pH 8.5: a) 50, 100, 200, 300 µg antennal; b) 110, 220, 440 µg head; c) 118, 236 µg tarsal/tibial; d) 100, 200 µg thoracic; e) 106, 212 µg wing; f) 50, 100, 200, 300 µg abdominal. Rf 100 is indicated by a dotted line or by the fastest moving protein band.

into an Rf 11 band for 106 µg and Rf 23 for 212 µg. Abdominal protein resolved an Rf 23 band from 50 µg applied and 33 for 100 µg, 57 for 200 µg and 66 for 300 µg. These abdominal bands illustrated a significant increase in mobility as compared to the other extracts. Deletion of the Triton from the gels resulted in a fragmentation of the major fraction (Figure 2a).

Electrophoreted antennal extracts in 3, 5 and 7% acrylamide gels, containing 0.6% Triton X-100, are illustrated in Figure 2b. As the acrylamide concentration was reduced, the Rf value of the major band was only slightly increased; however, the density of this band was progressively reduced, while the density of a proteinaceous band at the glycine front was progressively increased. 100 µg of antennal extract resolved into an Rf 26 band with the 3% acrylamide, 21 with the 5% and 19 with the 7%.

Under the above conditions, Triton X-100 aggregated, and maintained, most of the solubilized proteins in one heterogeneous fraction. The sieving effects of the different acrylamide concentrations remained high. This indicated a cross-linked proteinaceous structure, with subunit break-off running at the glycine front.

Electrophoresis of 0.6% Triton X-100 in 0.9% NaCl solubilized extracts in pH 7.1 gels (with or without Triton incorporation) and pH 7.1 reservoir buffers resolved several proteinaceous fractions. Employment of different acrylamide concentrations produced distinctive sieving effects on certain of these electrophoreted bands (Figure 2c). When the radiolabelling experiments of FERKOVICH and NORRIS[4] were repeated, except at pH 7.1, label was incorporated into band Rf 81 in 3.5% acrylamide, bands Rf 77 and 56 in 7% acrylamide and band Rf 21 in 14% acrylamide (Figure 2c)[7]. These observations demonstrated an apparent sieving action by the denser acrylamide gels on the proteins binding the labelled feeding inhibitor, menadione^{14}C.

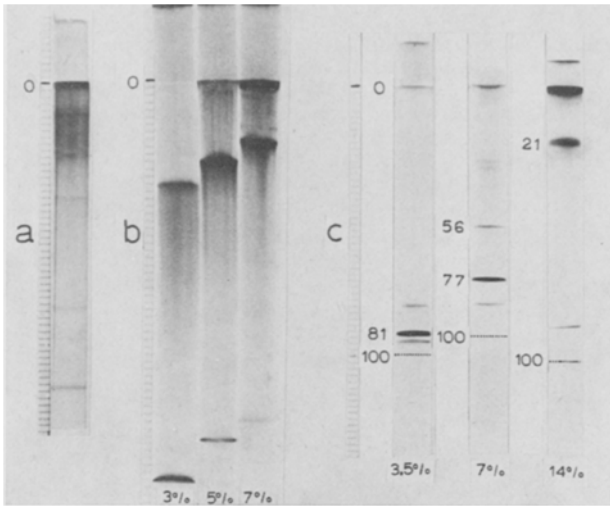

Fig. 2. Electrophoreted Triton-solubilized antennal proteins a) in gel not containing Trition X-100, pH 8.5; b) on different concentrations of acrylamide, pH 8.5; and c) in different concentrations of acrylamide, pH 7.1.

Resuspension of pellets in different concentrations of Triton X-100 resulted in altered protein solubilization. Folin analysis[6] and electrophoresis of samples indicated that a 1% solution of Triton in 0.9% NaCl solubilized less than 1/2, and a 5% solution solubilized less than 1/4 as much protein as did the 0.6% Triton extraction.

SDS electrophoresis has become an established means for the determination of protein molecular weights[8]; however, addition of SDS to our obtained proteinaceous extracts, as yet, has produced no banding with SDS electrophoresis.

Although Triton X-100 is commonly employed in the solubilization of pellet-bound proteins, our findings illustrated and emphasized certain effects which Triton can have on protein solubilization and fractionation. Experimental parameters regarding Triton treatment and use must be accurately defined and controlled to insure reproducible, meaningful results[9].

Resumen. Un agregado de proteínas solubilizadas en Triton X-100 fue resuelto como una banda por electroforesis a un pH de 8.5. En un pH de 7.1 estas fracciones no demuestran el efecto de agregación. Solamente electroforesis con *Tris*/glicina como amortiguador y concentraciones del detergente menor de 0.6% dieron resultados.

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Occurrence of Vesicles in Rabbit Seminal Plasma

The fertilizing capacity of rabbit spermatozoa recovered from the uterus of a doe is inhibited by a fast sedimenting component in seminal plasma from intact and vasectomized bucks that was isolated recently following centrifugation on sucrose density gradients[1]. Rabbit seminal plasma had been previously postulated to possess a factor of macromolecular dimensions capable of reversibly blocking sperm fertilizing capacity, when it was observed ultracentrifugation removed decapacitation activity in a few hours[2]. This report shows the inhibitor fraction consists of numerous small vesicles and, in addition, that another rapidly sedimenting seminal plasma fraction of higher density also contains vesicles.

Seminal plasma was collected with an artificial vagina from bucks of mixed strains, having proven fertility. Fresh semen samples with good sperm motility were centrifuged at $1000 \times g$ for 30 min and the seminal plasma aspirated. Rapidly sedimenting components were recovered from this fluid by employing discontinuous

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