Selective Staining of Rodent Acrosomes with Phosphotungstic Acid

(Selektive Färbung der Acrosomen von Nagetieren mit Phosphorwolframsäure)

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With 8 figures

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Summary

The acrosome of rodents, as well as the acrosomal granule, the head cap and the elastic materials fixed in glutaraldehyde and embedded in EPON, show a high degree of affinity for aqueous phosphotungstic acid (PTA). Under the electron microscope the reactive material shows up in strong contrast when fine sections have been floated on aqueous 3—5% PTA. In thick PTA-treated sections, the PTA can be easily located by light microscopy by subsequent staining with methyl green pyronin or UNNA's blue.

Zusammenfassung


Introduction

It is now known that in the course of mammal spermatogenesis the acrosome appears as a characteristic differentiation of the Golgi apparatus consisting of glycoproteins. The "periodic acid-fuchsin sulfuric acid" staining technique (Leblond, 1950) has proved one of the most suitable cytochemical procedures for study.

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ing the formation of the acrosome in rodents under the light microscope (LeBlond and Clermont, 1952). On the other hand, the “periodic thiosemicarbazide-silver proteinate” (Thierry, 1967) and “chromic acid-phosphotungstic acid” (Rambourg, 1969b and Rambourg et al., 1969) staining techniques, which show up the polysaccharides in electron microscopy, also stain the acrosomal material.

In this paper, we report the results of a phosphotungstic acid staining technique (PTA) in aqueous solution carried out on material included in EPON which enable us to show up the acrosomes of rodents selectively in both light and electron microscopy.

Material and Methods

Small pieces of adult mouse and rat testicles and ovaries were fixed in 2.5% glutaraldehyde in phosphate buffer at pH 6.9 for 1—2 hrs, dehydrated in alcohol and embedded in Epon 812.

Light microscopy. Thick sections (about 0.5—1 μm) mounted on slides were treated with 3—5% PTA in distilled water (about pH 2) for 2, 6 and 16 hrs at room temperature; then, after being washed with distilled water, the sections were stained with a 2% aqueous solution of methyl green at 60°C for 3—5 minutes. Other stains which, like methyl green, also precipitate in test tubes with PTA, such as pyronin and Unna’s blue, are also useful for bringing out the PTA deposit in thick sections.

Some testicles were fixed in Zenker and embedded in paraffin. Sections of these blocks were treated with 3% aqueous PTA for 2 hrs and then washed and stained with 0.02% pyronin in distilled water, dehydrated and mounted in Canada balsam.

Electron microscopy. Thin sections on Cooper grids were floated on 3—5% PTA in distilled water at room temperature for 20—30 minutes. After two washings in distilled water, the grids were observed under a Philips 300 electron microscope.

Some specimens fixed in glutaraldehyde were postfixed in 1% osmium tetraoxide in phosphate buffer for 1—2 hrs and the corresponding sections were stained with uranyl acetate and lead citrate as usual.

Results

Light microscopy. The study of thick sections stained with PTA-methyl green reveals that in the seminiferous tubules of both species the most strongly stained structure is the acrosome. In the young spermatids (fig. 1) we find both the acrosomal granule and the head cap stained (‘‘cap phase’’). In the spermatids in process of elongation (‘‘acrosome phase’’) the acrosome also shows up strongly stained (fig. 2), adopting the forms characteristic of the species concerned. Thick sections treated exclusively with PTA or with methyl green do not show any positive reaction in the acrosome, whereas any of the colouring materials that precipitate with an aqueous solutions of PTA (such as methyl green, pyronin and Unna’s blue) enable us to observe these structures. The same possibilities of observation are afforded in the case of the acrosome with different concentrations and with different PTA-treatment periods.

In addition to the staining of the acrosome with this technique, a similar staining intensity is observed in the elastic fibres of the arteries, and very slight positive effects in the basal membranes of the seminiferous tubules. But the pellucid mem-
brane of oocytes with growing follicles turns out completely negative with this technique.

The sections of material stained with Zenker and included in paraffin, and those stained with pyronin, also show a strong staining effect on the acrosome, though it is less selective.

Electron microscopy. At a low magnification, the thin sections of seminiferous tubules that have been stained with 5% aqueous PTA show that the structures most strongly contrasted are the acrosomes (fig. 6). The acrosomal granule and the head cap in the "cap phase" of spermatogenesis show up strongly stained (fig. 3 and 4). At this stage, some of the vesicles near the head cap prove to be lightly stained, while the cisternae of the Golgi apparatus show practically no staining effect (fig. 3). In all cases we observe that the PTA-reactive material is unevenly distributed inside the head cap. As spermatogenesis proceeds, the head cap becomes increasingly flattened out against the nucleus, and as its membranes come closer together the entire structure (acrosome, fig. 5) appears to become progressively thinner and more strongly contrasted (fig. 6, 7 and 8).

Discussion

Up to the present, comparatively few cytochemical studies of the acrosome have been published. We may agree with Leblond and Clermont that the characterization of the 1 and 2 glycol groupings by the "periodic acid fuchsin sulfuric acid" technique gives grounds for suggesting the presence of polysaccharides in rodent acrosomes, which are neither metachromatic nor digestible with amylase.

It is now thought that PTA interacts selectively with positively charged groups in several polymers, and therefore fundamentally stains the proteins (Silverman and Glick, 1969; Quintarelli et al., 1971a and b; Scott, 1971). However, the absence of results comparable to those obtained with alcoholic PTA staining (Sheridan and Barnett, 1969) in rat testicles (Esponda and Giménez-Martín, 1972) justifies us in suggesting that the selective staining of the acrosome with aqueous PTA in EPON-embedded testes implies more specific interaction with the PTA reactive components.

Most chemical evidence support the view that PTA has a specific affinity for polysaccharides in strongly acidic solutions (see Hayat, 1970). Several authors have reported the selective staining of complex carbohydrates and of glycoproteins by treating sections of glycol or hydroxypropyl methaerylate embedded material with aqueous PTA only, or mixture with hydrochloric or chromic acid (Pease, 1966; Rambourg, 1967; Marinozzi, 1968; Rambourg, 1969a and b; Pelletier, 1971; and Flechon, 1971). The components stained with PTA are distributed, according to these authors, in the glycogen, the Golgi apparatus, lysosomes, basal membranes, elastic fibres, acrosome and pellicid membrane.

Fig. 1 and 2. Thick sections of Epon embedded mouse testis stained with 5% aqueous PTA-2% methyl green. The reaction of the acrosome can be clearly observed.

Fig. 3 and 4. The acrosomal granule and the material contained in the acrosomal vesicle appear strongly stained. Short arrow shows the unstained Golgi cisternae and long arrow indicates a moderate contrast of some vesicles. N=nucleus. Fig. 3: 25,000× (Rot). Fig. 4: 31,000× (mouse).
Fig. 5. Rat spermatids. Arrows show acrosomes. N = nucleus. Material fixed in glutaraldehyde-osmium, embedded in Epon stained with uranyl-lead. 15,000×.

Fig. 6. Mouse elongated spermatids show high contrast in the acrosome after staining with 3% aqueous PTA. 10,800×.

Fig. 7 and 8. Acrosomes of rat spermatids high contrasted after PTA reaction. N = nucleus. In fig. 8 arrows show the two joined membranes. Fig. 7: 28,000×. Fig. 8: 41,000×.
The protonation of hydroxyl groups at a very low pH enables the PTA reaction to take place and, like hydroxyl polymerase, polysaccharides can also react with PTA (QUINTARELLI et al., 1971a and b; SCOTT, 1971). In the conditions used here (embedding in EPON), the PTA evidences even greater selectively for aerosomal and elastic materials than that evidenced by hydrophilic inclusion methods.

The rapidity and ease with which this procedure can be used, as well as the possibility of distinguishing the PTA-reactive material at the light microscopy level, afford us a technique which may be particularly useful for studies of spermiogenesis in both light and electron microscopy.

References


