

Distribution of acid glycosidases in the male genital tract of the pheasant

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SUMMARY

The distribution of the activity of seven acid glycosidases in the reproductive organs of the pheasant (*Phasianus colchicus*) was studied. The study was carried out on seven mature birds at the age of 11 months during the reproductive season (May). Significant ($p < 0.01$) differences in acid glycosidase activity dependent on enzyme origin were observed. Generally, the highest activity of acid glycosidases was found in the epididymis, intermediate in the ductus deferens and the lowest in the testes. Exceptionally, α -mannosidase had the highest activity in the ductus deferens. Anion-exchange chromatography elution profiles of most enzymes from the tested reproductive organs were similar, however evident differences were observed for β -mannosidase forms. *Reproductive Biology* 2006 **6** Suppl. 2:65–73.

Key words: pheasant, reproductive system, acid glycosidases

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INTRODUCTION

Acid glycosidases [EC 3.2.1] are a group of hydrolytic enzymes that catalyze the breakdown of terminal oligosaccharide units of glycoproteins and glycolipids. Lysosomal and acrosomal glycosidases of an acid range of pH 3.5-5.5 are characterized by high substrate specificity [5, 9]. Acid glycosidases are common in tissues of the male genital tract and semen of mammals [6, 8, 18]. Due to, regulated by sex hormones [7], the high activity of acid glycosidases in the male genital tract, these enzymes are certainly implicated in the male fertility, but their specific roles are not fully understood yet [3, 4, 19].

Acid glycosidases were found to be particularly active in mammalian epididymides compared to the other parts of the genital tract [8, 14, 15]. In birds, excurrent ducts consist of paired rudimentary epididymides and ductus deferentia. In the epididymis, avian spermatozoa undergo the maturation process, in which they mostly acquire motility [16]. The ductus deferens, especially its terminal part, is the main extragonadal reserve of spermatozoa [13].

The activity of acid glycosidases in the reproductive tract of domestic fowl was investigated in roosters [1, 10], turkeys [10] and seasonally breeding ganders [11]. This study is the first to explore the occurrence of acid glycosidases in the genital tract of the pheasant.

MATERIALS AND METHODS

The study was carried out on seven mature pheasant cocks in May during the reproductive season. The birds were obtained from a pheasantry and were kept under the same environmental and feeding conditions. Reproductive organs were collected soon after slaughter of the birds (11 months old with similar body weights 1285 ± 250 g), and stored at -25°C until analyses. To determine the activity of enzymes, tissues were homogenized in 1% NaCl at the ratio of 1:4 (w/v) using VirTis HandiShear Homogenizer (25 000 rpm for 3×15 s at 0°C). The homogenates were centrifuged at $12\ 000 \times g$, while the supernatant was used for the determinations.

The activities of the following acid glycosidases were determined: N-acetyl- β -D-hexosaminidase (β -HEX), α -D-galactosidase (α -GAL), β -D-galactosidase (β -GAL), α -D-mannosidase (α -MAN), β -D-mannosidase (β -MAN), α -L-fucosidase (α -FUC) and α -D-glucosidase (α -GLU). Enzymatic assays of glycosidase activities were performed according to the method described by Barrett and Heath [2] based on absorbance measurements at 400 nm of enzymatically released *p*-nitrophenol (*p*-NP) from suitable *p*-NP-glycosides (Sigma USA). Briefly, incubation mixtures containing an appropriately diluted enzyme sample, 1 mM proper *p*-NP substrate and 0.1 M citrate buffer at optimum pH (3.7 for β -GAL; 4.5 for β -HEX, α -MAN, β -MAN and α -GAL; 5.0 for α -FUC and α -GLU) were incubated at 37°C. The reaction was terminated by the addition of trichloroacetic acid (2% final dilution). One unit (U) is defined as the enzyme activity hydrolyzing 1 μ mol of substrate per min at 37°C at optimal pH conditions. Specific activities are expressed as units of enzyme activity per milligram protein (U/mg). Protein was assayed using the biuret method and serum bovine albumin as the standard [17].

The enzymes were separated into multiple forms using anion-exchange chromatography. A column of 7 cm³ anion exchanger Macro-Prep[®] High Q Support (High Q) attached to the BioLogic-LP (Bio-Rad) system, was equilibrated with 25 mM histidine-HCl buffer (pH 6.0). Samples of tissue homogenates (0.5 ml) were dialysed 24 h at 4°C against the same buffer before column chromatography. Unretained proteins were eluted with the column buffer and then a linear gradient of NaCl (0-0.5 M) was applied. Finally the column was eluted with 1 M NaCl in the same buffer. For statistical evaluation of the results, one-way analysis of variance was used followed by LSD test (Statistica, StatSoft Inc., Tulsa, OK, USA).

RESULTS

In the genital tract of the pheasant, the activities of β -HEX, β -GAL, α -MAN and β -MAN were high and those of α -GAL, α -FUC and α -GLU were much lower (tab. 1). The activity of all the enzymes analysed was

found to be much higher ($p < 0.01$) in the epididymal homogenate than in the testicular (by 3-20 times) and ductus deferens homogenates (by 1.5-3 times). Exceptionally, α -MAN showed the highest activity in the ductus deferens.

Optimum pH of the enzymes present in testes and epididymides were identical: 3.7-4.0 for β -GAL, 4.25 for β -HEX, 4.5 for α -MAN, β -MAN and α -GAL, and 5.0-5.25 for α -FUC and α -GLU.

Anion ion-exchange chromatography usually enabled the enzymatic activities to be separated into two forms: the major (bound to the anion

Table 1. Specific activity (mU/mg protein) of acid glycosidases in the male genital tract of sexually active pheasants; means \pm SD (n=7)

Enzyme	Testes	Epididymis	Ductus deferens
β -HEX	7.140 \pm 0.803 ^a	36.682 \pm 1.057 ^b	25.817 \pm 1.375 ^c
β -GAL	1.916 \pm 0.282 ^a	5.958 \pm 0.253 ^b	2.380 \pm 0.405 ^c
β -MAN	1.388 \pm 0.123 ^a	26.760 \pm 3.156 ^b	15.362 \pm 0.254 ^c
α -MAN	1.280 \pm 0.160 ^a	28.204 \pm 1.120 ^b	39.796 \pm 1.553 ^c
α -GAL	0.439 \pm 0.154 ^a	3.293 \pm 1.491 ^b	0.827 \pm 0.001 ^c
α -FUC	0.373 \pm 0.083 ^a	1.059 \pm 0.077 ^b	0.507 \pm 0.026 ^a
α -GLU	0.315 \pm 0.039 ^a	0.810 \pm 0.042 ^b	0.675 \pm 0.044 ^b

^{a,b,c} means in the same row with different superscripts differ at $p < 0.01$

exchanger) and the minor (unbound). The major form, which accounted for approximately 80-90% of total activity, was usually eluted at conductivity of 6-8 mS. Elution profiles of acid glycosidases such as β -HEX (fig. 1), α -GAL (not shown) and α -GLU (not shown), which originated from different parts of the genital tract, were identical.

An additional form of α -FUC, specific for the ductus deferens and eluted at conductivity of 17 mS, was observed (fig. 1). For β -GAL, two forms that bound to the exchanger were observed but they differed in their charge depending on the part of the genital tract (fig. 1). The greatest variety of forms was found for β -MAN depending on the origin of the enzyme. A bound (65%) and unbound form (40%) occurred in the elution profile of β -MAN from the testes, a form not bound to the exchanger (65%) dominated in the epididymis,

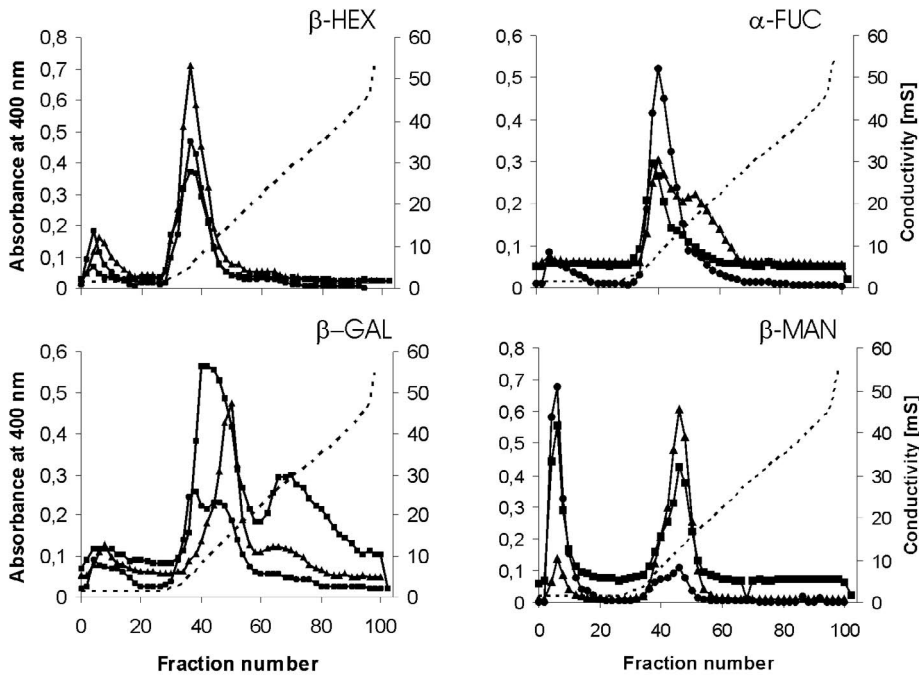


Figure 1. Elution profiles of acid glycosidases from testes (—■—), epididymides (—●—) and ductus deferens (—▲—) obtained by anion exchange chromatography. Results for β -N-acetylhexosaminidase (β -HEX), α -fucosidase (α -FUC), β -galactosidase (β -GAL) and β -mannosidase (β -MAN) are shown.

and a bound form (80%) prevailed in the ductus deferens (fig. 1). The bound form was eluted from the column at conductivity of 11-12 mS approximately.

DISCUSSION

This is the first study to describe the occurrence of acid glycosidases in the genital tract of the pheasant. The optimum pH of the acid glycosidases from pheasant testes and epididymides ranged from 3.7 to 5.25 and was highly similar or identical to the enzymes found in the genital tract of wild pheasants [12], other domestic fowl [10] and different species of mammals [9].

The highest epididymal and testicular activity was shown for β -HEX, its activity being five times higher in epididymides and four times higher

in the ductus deferens than in the testes. The second most active β -GAL was much less active than β -HEX (4, 6 and 11 times lower in testes, epididymides and ductus deferens, respectively). β -GAL activity was three times higher in epididymides than in testes, and 25% higher in the ductus deferens than in the testes as well. The occurrence of β -HEX and β -GAL in the genital tract of birds was described for cocks [1, 10], turkeys [10], ganders [11] and Japanese quails [Józefczyk, unpublished]. The activity of both glycosidases in the epididymides of domestic fowl was shown to be much higher than in the testes. For example, Bamberg et al. [1] found β -HEX activity to be 15 times higher, and β -GAL activity to be 2.5 times higher in cock epididymides compared to testes. They also showed that β -HEX activity is 2.5 times higher in the ductus deferens than in the testes but for β -GAL they found the lowest activity in ductus defferens.

A particularly high increase in activity was found for α - and β -MAN in epididymides (about 20-fold) and the ductus deferens (11- and 31-fold for β - and α -MAN, respectively). A several-fold increase in the activity of both mannosidases in the epididymis compared to testes was shown for cocks and turkeys [10]. While in Japanese quails, epididymal activity was 10 and 30 times higher than testicular activity for α - and β -MAN, respectively [Józefczyk, unpublished], similar to β -MAN from ganders' epididymides [11]. Of the other enzymes, the increase in epididymal compared to testicular activity was 8-fold for α -GAL and 3-fold for α -FUC. Similar results were obtained for ganders [11], but for Japanese quail, this increase was much more pronounced than that described in the present study (by 39 and 19 times for α -GAL and α -FUC, respectively) [Józefczyk, unpublished]. Whereas in turkey epididymides, the activity of both enzymes was slightly higher (20-30%) than in testes, and in cocks, α -FUC activity was 40% lower in epididymides than in testes [10].

Elution profiles for multiple forms of particular acid glycosidases found in the analysed parts of the genital tract were very similar, if not identical. The activity of all the examined enzymes was separated into the unbound (minor) fraction and the dominant fraction that bound to the anion exchanger, eluted with a gradient of 60-160 mM NaCl. For all the enzymes, these

fractions had isoelectric points below pH 6.0, while forms with the lowest charge were found for β -GAL and α -FUC. For β -MAN from epididymides, the forms with pI >6.0 were predominant. The occurrence of multiple forms of glycosidases with pI of 6.5-4.2 in avian testes and epididymides was shown repeatedly using the chromatofocusing method [10, 11].

It is generally believed that the maturation of spermatozoa is completed when the spermatozoa reach the distal segment of cauda epididymis. However, in species in which spermatozoa are stored in the ductus deferens for some time before they are ejaculated, the maturation may continue or even be completed in the ductus deferens. Yanagimachi et al. [22] showed that Chinese hamster spermatozoa from the ductus deferens must be more competent for fertilization than epididymal spermatozoa.

It is assumed that in mammalian epididymides, spermatozoa undergo the process of maturation in which glycoconjugates on the surface of spermatozoa are modified due to the combined action of glycosidases, glycotransferases and proteases [21]. The possibility of enzymes with acid pH optima being active in the neutral environment of the epididymal fluid was explained by Tulsiani et al. [20], who showed that acid β -galactosidase of epididymal lumen shows activity at neutral pH toward natural substrates. Spermatozoa in epididymides stay much shorter in birds than in mammals, the value for cocks being 72 h [13]. However, the high temperature in avian epididymides can considerably accelerate the enzymatic processes associated with the modification of oligosaccharide chains on the surface of spermatozoa, in which acid glycosidases may take an active part.

The highest activity of acid glycosidase (especially α - and β -MAN and α -FUC) in the distal part of the pheasant reproductive tract and multiple forms secreted by the epididymis and the ductus deferens can support the hypothesis that both epididymides and the ductus deferens of birds are the site of sperm maturation, which results in the increased motility of spermatozoa. The presence of highly active acid glycosidases in the excurrent ducts suggests the role of these enzymes in sperm maturation in birds. The specific functions of acid glycosidases in sperm maturation require further studies.

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