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Glycocomposition of the apocrine interdigital gland secretions in the fallow deer (*Dama dama*)

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ABSTRACT

The secretions of the tubular interdigital glands were investigated by conventional (Periodic-Acid Schiff, Alcian-Blue at different pH, Low Iron Diamine and High Iron Diamine) and lectin (Con-A, UEA-I, LTA, WGA, GSA-II, GSA-IB4, SBA, PNA, ECA, DBA, MAL-II and SNA) histochemical methods in adult males and females of different age of fallow deer during the breeding season. Sialidase digestion and deacetylation pre-treatment were also employed in conjunction with lectin histochemistry.

The glandular epithelium consisted of a single layer of low columnar cells with typical apical protrusions. No substantial differences of the above histochemical staining in relation to sex and age were observed. Conventional histochemical staining revealed that the interdigital glands secreted neutral glycoproteins whereas acidic glycocomponents did not seem to be present. Lectin histochemical technique allowed us to disclose a great heterogeneity of glycoproteins with *N*- and *O*-linked oligosaccharides containing α-D-Man/α-D-Glc, GlcNAc, α-Fuc, terminal β-D-Gal-(1-3)-D-GalNAc, -D-Gal-(1-4)-D-GlcNAc, α-Gal and β-GalNAc residues. β-GalNAc and disaccharide β-D-Gal-(1-3)-D-GalNAc were also found as subterminal to sialyl moieties. The lack of sexual and age-related differences in the glucidic content of the glandular secretions seems to indicate that the glycoderivatives may play only an accessory role in the production of odoriferous signals in fallow deer.

1. Introduction

In Italy, fallow deer (*Dama dama*) is the most common cervid kept in national parks, reserves and extensively managed farms (Randi, 2005). These cervids are seasonal breeders: the breeding season, known as the rut, takes place in the fall, beginning in mid-September and continuing into November, but the peak breeding activity takes place in October. Fallow deer populations show a variety of mating systems, ranging from low-fidelity territorial/follower strategy (Moore et al., 1995) to lekking and deer harems (Clutton-Brock et al., 1988), but variance in mating success is generally very high (Moore et al., 1995; Clutton-Brock et al., 1988; Apollonio et al., 1989). Females reach sexual maturity at 16 months whereas bucks are mature sexually at 14 months but rarely compete successfully in rutting until several years later (Chapman and Chapman, 1970, 1980).

As in other wild ungulates, the communication of reproductive information in fallow deer is thought to be accomplished by odours associated with skin glands localized to specific areas, such as infraorbital, tarsal and interdigital areas (Osborn et al., 2000). The interdigital glands belong to a group of skin apocrine glands, varied in morphology, described in several Artiodactyla, among them in various species of deer (Wood, 1999, 2003; Wood et al., 1995a, b; Reiter et al., 2003), in the Japanese serow, *Capricornis crispus* (Atoji et al., 1988), in the impala, *Aepyceros melampus* (Welsch et al., 1998) and in the Asian elephant, *Elephas maximus* (Lamps et al., 2001). As regards their function, these glands are considered scent glands producing odorous signals and pheromones that play important biological roles in the conspecific chemical communication, such as active territorial demarcation and in the expression of social behaviour (Robertshaw, 1987; Epple et al., 1993). Several studies have been carried out in order to identify the chemicals in the secretions of skin glands, including foot or interdigital glands of reindeer, *Rangifer tarandus* (Brundin et al., 1978; Andersson et al., 1979; Brundin and Andersson, 1979), white-tailed deer, *Odocoileus virginianus* (Gassett et al., 1996; Wood, 1999), and black-tailed deer, *Odocoileus hemionus columbianus* (Wood et al., 1995a, b), the forehead gland of white-tailed deer (Gassett et al., 1997), the tarsal gland of black-tailed deer (Müller-Schwarze et al., 1978) and reindeer (Andersson et al., 1975), the preorbital gland of reindeer (Sokolov et al., 1977; Andersson, 1979), metatarsal gland of sika deer, *Cervus nippon* (Wood, 2003), the caudal gland of reindeer (Müller-Schwarze et al., 1977), and the tail gland of red deer, *Cervus elaphus* (Bakke and Figenschou, 1983). Similarly to other odoriferous glands, the major constituents of the scent

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material secreted by the interdigital glands are volatile elements such as alkanes, isoalkanes, ketones and aldehydes, already biochemically characterized in a range of species (Reiter et al., 2003; Wood, 2003). However, among the secretory products of some scent glands, also non-volatile substances, such as glycoconjugates with different terminal sugars have been detected by means of lectin histochemical techniques (Atoji et al., 1988; Aoki-Komori et al., 1994; Welsch et al., 1998). Lectins are specific carbohydrate binding proteins of non-immune origin that agglutinate cells or precipitate polysaccharides or glycoconjugates, which have been widely used as histochemical probes for localizing and characterizing specific sugar residues or oligosaccharide sequences in cells and tissues (Spicer and Schulte, 1992).

Carbohydrates are involved in many biological activities and they are important key substances for the functional properties of the secretion of many exocrine glands (Damjanov, 1987; Spicer and Schulte, 1992).

To our knowledge, there is a lack of information on the glycocomposition of the secretory products of the fallow deer interdigital glands. In the present study, we first examined the morphology of the interdigital glands and then applied lectin histochemistry combined with enzymatic digestion and chemical treatments to it for analyzing the presence and distribution of the carbohydrate binding sites in these glands. In order to better understand some aspects of the biology of the fallow deer, possible sex- and age-related differences in the lectin affinity of the interdigital glands were also investigated.

2. Materials and methods

2.1. Animals

The whole interdigital glands of both fore and hind legs were collected from adult male ($n = 6$; aged from 18 months to 5-year-old) to adult female ($n = 6$; aged from 2 to 8-year-old) fallow deer during the breeding season (October–November). These skin samples were excised immediately after death of the animals, which were regularly slaughtered in an authorized abattoir for wild ungulates following principles of animal care and specific national laws. After gross dissecting, the interdigital glands were drawn and immediately fixed as follows.

2.2. Tissue preparation

The specimens of both fore and hind legs were immediately fixed in Carnoy's fluid for 24 h and post-fixed in a solution of 2% calcium acetate and 4% paraformaldehyde (1:1 v/v) for 3 h at room temperature (Menghi, 1984). They were then routinely dehydrated in graded series of alcohols, cleared in xylene and subsequently embedded in paraffin.

Serial sections 5 μm thick were mounted on Superfrost Plus slides (Bio-Optica, Milano, I) and subjected to conventional and lectin histochemical staining.

2.3. Conventional histochemical staining

The sections were stained with the following methods: Periodic-Acid Schiff (PAS) to detect vicinal hydroxyls, Alcian-Blue (AB) pH 2.5 and Low Iron Diamine (LID) to demonstrate acidic groups and AB pH 1.0, AB pH 0.5, and High Iron Diamine (HID) to discriminate sulphate groups (Pearse, 1985).

2.4. Lectin histochemistry

The specimens were processed for lectin histochemistry according to the procedures that were described previously by Parillo and Verini Supplizi (2008).

Table 2
Lectin binding patterns in the interdigital glands of fallow deer[a]

Staining and treatments	Glandular cells	Apical cytoplasmic protrusions
PAS	2	2
AB pH 2.5 and LID	0	0
AB pH 1 and HID	0	0
AB pH 0.5	0	0
Con-A	3	3
LTA	3	3
UEA-I	2	3
WGA	1	2
Sialidase/WGA	1	2
PNA	1	2
Sialidase/PNA	2	3
SBA	1	1
Sialidase/SBA	2	2
ECA	1	2
Sialidase/ECA	1	2
GSAIB4	1	2
Sialidase/GSAIB4	1	2
DBA	0	0
Sialidase/DBA	0	0
GSA-II	0	0
Sialidase/GSA-II	0	0
SNA	1	2
MAL-II	1	2

[a] Evaluations were performed by attributing scores from 0 to 3 according to the following criteria: the reactivity was classified as absent (score of 0) when there are non-glandular cells or apical protrusions per high-power field (HPF, using 400× magnification), weak (score of 1) for a few cells (1–19) per HPF, moderate for a discrete number of cells (20–49) per HPF, and strong (score of 3) for numerous cells (50–99) per HPF.

Table 1
Lectins used and their carbohydrate specificities

Source of lectin	Acronym	Carbohydrate specificity[a]	Inhibitory sugars[b]	Lectin concentration (μg/ml)
Canavalia ensiformis	Con-A	α-D-Man > α-D-Glc	α-D-Methylman	20
Triticum vulgaris	WGA	GlcNAc > sialic acid	D-GlcNAc	10
Griffonia simplicifolia	GSA-II	α and β GlcNAc	D-GlcNAc	50
Glycine max	SBA	α-D-GalNAc > β-D-GalNAc	D-GalNAc	10
Arachis hypogaea	PNA	β-D-Gal-(1 → 3)-D-GalNAc	D-Gal	40
G. simplicifolia IB4	GSA-IB4	α-D-Gal	D-Gal	20
Ricinus communis	ECA	β-D-Gal-(1 → 4)-D-GlcNAc	D-Gal	50
Dolichos biflorus	DBA	α-D-GalNAc	D-GalNAc	10
Lotus tetragonolobus	LTA	α-L-Fuc	L-Fuc	20
Ulex europaeus	UEA-I	α-L-Fuc	L-Fuc	20
Sambucus nigra	SNA	NeuAc(α2,6)Gal/GalNAc	NeuAc	100
Maackia amurensis	MAL-II	NeuAc(α2,3)Gal	NeuAc	100

[a] β-D-Gal, β-D-galactose; α-D-Gal, α-D-galactose; D-GalNAc, D-*N*-acetylgalactosamine; β-D-GalNAc, β-D-*N*-acetylgalactosamine; α-D-GalNAc, α-D-*N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; α-D-Man, α-D-mannose; α-D-Glc, α-D-glucose NeuAc, *N*-acetylneuraminic acid.
[b] α-D-Methylman, α-D-methylmannose.