Commentary

The structure of the O-linked carbohydrate chain of bovine seminal plasma protein PDC-109 revised by ^{1}H-NMR spectroscopy
A correction

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PDC-109, a 13-kDa protein [1], is the major secretory product of the seminal vesicles of the bull (*Bos taurus*) and constitutes the most abundant protein of seminal plasma (16–25 mg/ml) [2]. It possesses heparin- and phosphorylcholine-binding activities [3] and coats the spermatozoa surface at ejaculation [4], enhancing their fertilizing capability [5]. PDC-109 has a single O-glycosylated residue (threonine 11) [4]. Carbohydrate composition, mass spectrometric, and lectin mapping analyses suggested the Neu5Ac(α2–6)-Gal(β1–3)-GalNAc structure for the carbohydrate chain of bovine PDC-109 [4]. Here, we report the revision of the oligosaccharide structure of PDC-109 after ^{1}H-NMR spectroscopic analysis.

PDC-109 was isolated from the seminal plasma of Holstein bulls by chromatography on a DEAE-Sephadex A25 equilibrated with 10 mM Tris/HCl, 1 M NaCl, 5 mM EDTA, 0.025% NaN_3, pH 7.4. PDC-109 was eluted with column buffer containing 10 mM *o*-phosphorylcholine. O-linked carbohydrate chains were released from PDC-109 (200 ml) by alkaline borohydride treatment (β-elimination) [6] with 50 ml of 0.1 M NaOH, 1 M $NaBH_4$ for 48 h at 37°C in the dark and under a nitrogen atmosphere. After centrifugation (3000 rpm, 20 min), the supernatant was acidified to pH 6 with 4 M acetic acid at 0°C, applied to a 11×2.5 cm Dowex 50X8 (H^+ form) column, washed with ~200 ml cold water, and the eluate lyophilized. Boric acid was removed by co-evaporation with methanol. The remaining material was fractionated on a BioGel P2 column (46×1.6 cm, BioRad) eluting with 5 mM NH_4HCO_3. Fractions, monitored at 206 nm, were tested for hexose content by the orcinol/H_2SO_4 method. The major carbohydrate-containing fraction was purified by gel filtration chromatography on a 46×1 cm BioGel P4 column (BioRad). Carbohydrate-containing fractions were repeatedly treated with D_2O (99.96 atom% D, Isotec Inc.) with intermediate lyophilization. High-resolution 500-MHz ^{1}H-NMR spectra were recorded with a Bruker AMX-500 spectrometer at a probe temperature of 300 K. Acetone (δ 2.225 ppm) was used as internal standard [7].

Monosaccharide analysis of O-linked carbohydrates released from PDC-109 by β-elimination demonstrated that GalNAc was completely converted into GalNAc-ol (Table 1). The little amount of Gal-ol found (~10% of total Gal) indicated that peeling may have occurred during the β-elimination procedure [8]. Fractionation on BioGel P2 yielded six fractions, denoted P2-I to P2-VI. Fraction P2-V contained free monosaccharide alditols. Fraction P2-IV contained neutral material and ^{1}H-NMR spectroscopy proved the structure to be Gal(β1–3)-GalNAc-ol [9]. Fraction P2-I was further purified on BioGel P4, and four subfractions, designated P4-I to P4-IV, were recovered. Fractions P4-I and P4-II did not contain carbohydrate. Fraction P4-IV contained a small amount of free sialic acid. ^{1}H-NMR spectroscopy of fraction P4-III clearly demonstrated the structure Neu5Ac(α2–3)-Gal(β1–3)-GalNAc-ol: the β-Gal residue was identified by the H-1 and H-4 signals at δ 4.547 ($J_{1,2}$ 7.9 Hz) and δ 3.931 ppm, respectively. The Gal H-3 was observed at δ 4.122 ppm. The Gal(β1–3)-GalNAc-ol core was characterized by the H-2 and H-5 signals of GalNAc-ol at δ 4.389 and 4.188 ppm, respectively. The NAc singlet of GalNAc-ol was observed at δ 2.046 ppm. The structural-reporter groups of Neu5Ac, namely NAc signal at δ 2.034 ppm, H-3a at δ 1.800 ppm, and H-3e at δ 2.773 ppm, indicated an α-Neu5Ac residue linked to C3 of the galactose residue.

Table 1
Monosaccharide analysis of PDC-109 and derived fractions

Monosaccharide	PDC-109		BioGel P2			BioGel P4	
	native	after β-elimination	P2-I	P2-IV	P2-V	P4-III	P4-IV
Gal	1.0	0.9	1.1	1.1	±	1.3	–
Gal-ol	–	0.1	0.1	0.1	1.1	–	±
GalNAc	0.9	–	–	–	–	–	–
GalNAc-ol	–	1.0	1.0	1.0	1.0	1.0	–
Neu5Ac	1.1	1.1	1.2	±	–	1.2	1.0

Values are given in mol saccharide per mol protein. ± means present, but less than 0.1.

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This study confirms that the major (>80%) carbohydrate structure of PDC-109 is a trisaccharide and, in addition, unambiguously establishes that the anomery of the *N*-acetyl neuraminic acid-galactose linkage is α2–3 rather than α2–6.

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