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Analysis of Acid-Soluble Glycogen in Pork Extracts of Two *PRKAG3* Genotypes by ^{1}H Liquid-State NMR Spectroscopy and Biochemical Methods

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ABSTRACT: Meat extracts with acid-soluble glycogen (macroglycogen) from M. longissimus dorsi of carriers and noncarriers of the *PRKAG3* mutation (*RN*$^-$ and *rn*$^+$ genotype) were analyzed by both ^{1}H liquid-state NMR spectroscopy and a biochemical method. The ^{1}H NMR analysis revealed that shorter polymers (dimers, trimers, etc.) of α-1,4-linked glucose were generated 24−48 h post-mortem. This is not possible to elucidate with the biochemical method, by which only the total amount of hydrolyzed glucose residues is determined. The shorter polymers were primarily formed in carriers of the *PRKAG3* mutation, suggesting different post-mortem glycogen degradation mechanisms in the two genotypes.

KEYWORDS: glycogen, ^{1}H nuclear magnetic resonance (^{1}H NMR), enzymatic analysis, pork, RN-gene, *PRKAG3* mutation

INTRODUCTION

Glycogen is the cellular energy storage molecule in animals and serves the same purpose as starch does in plants. Both starch and glycogen are α-linked polymers of glucose, but whereas starch is composed of linear amylose and more branched amylopectin, glycogen is a large branched polymer of glucose units, similar to amylopectin, built up on a core protein, glycogenin.[1]

Glycogen has been divided into two molecular subgroups according to its solubility in trichloroacetic acid or perchloric acid: acid-insoluble glycogen, called proglycogen, with a protein content of about 10% (molecular weight 400 kDa) and acid-soluble glycogen, called macroglycogen (molecular weight 10^7 Da) with only 0.35% protein content.[1] Methods for the analysis of glycogen in biological tissues are traditionally based on the hydrolysis of glycogen to glucose units either by acid or enzymatic hydrolysis. Total glycogen as well as pro- and macroglycogen have reliably been measured by both of these methods.[2] However, these biochemical methods do not reveal the actual size and size distribution as well as the configuration or structure of the glycogen molecules. By ^{1}H liquid-state nuclear magnetic resonance (NMR) spectroscopy hydrogens in various chemical environments in solution can be identified and quantified, for example, the anomeric proton involved in the α-1,4 glucosidic bond in glycogen. Previously, this approach has been used for structural analysis of rabbit liver glycogen.[3]

Recent microscopy studies[4,5] demonstrated that two kind of particles can be present in glycogen: β-particles having diameters in the range of 10−30 nm and supramolecular complexes of β-particles, the so-called α-particles, having diameters up to 300 nm. The α-particles are so far only observed in liver glycogen, whereas glycogen from skeletal muscles contain only β-particles.[5] Similar to amylopectin, the glucose units in glycogen are connected by linear chain α-1,4 and branched α-1,6 glucosidic bonds. The glycogen molecule is formed by two different kinds of chains, A- and B-chains.[4] The A-chains are not branched, having only α-1,4 bonds, whereas the B-chains are branched, each of them with two branching points with α-1,6 bonds, which create new either A- or B-chains. There are four glucose residues between the branches and a tail of approximately four residues after the second branch in the B-chains. The glycogen molecule is spherical and organized into concentric tiers. Every B-chain is in the inner tiers, and every A-chain is within the outer tier. Each A- or B-chain has 12−14 glucose residues, and there are 12 tiers in a molecule. The number of chains in any tier is twice that of the previous one, as a consequence of two branching points at each chain.[6]

The dominant *PRKAG3* mutation (previously known as the *RN*$^-$ mutation) in pigs of the Hampshire breed results in very high glycogen content, especially in glycolytic muscles, compared with the wild type Hampshire pigs.[7] Furthermore, the post-mortem pH decline has been shown to be slightly faster and the ultimate pH lower in M. longissimus dorsi (LD) of carriers of the *PRKAG3* mutation compared with noncarriers.[8−11] Essén-Gustavsson et al.[12] studied post-mortem degradation of total glycogen in LD of carriers and noncarriers of the *PRKAG3* mutation using a biochemical method. As expected, they found a higher glycogen content in carriers compared with noncarriers of the *PRKAG3* mutation, but the amount of glycogen degraded from 10 min to 24 h post-mortem did not differ between the genotypes. Samples of LD from the same pigs as in the study of Essén-Gustavsson et al.[12] were in the present study homogenized with perchloric acid and centrifuged, and the soluble glycogen in the supernatant (macroglycogen) was then analyzed

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