Analytical Biochemistry 529 (2017) 117–126

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Technical and experimental features of Magnetic Resonance Spectroscopy of brain glycogen metabolism

Ana Francisca Soares [a], Rolf Gruetter [a, b, c, d], Hongxia Lei [b, c, *]

[a] Laboratory for Functional and Metabolic Imaging (LIFMET), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
[b] Department of Radiology, University of Genève (UNIGE), Switzerland
[c] Center for Biomedical Imaging (CIBM), Lausanne, Switzerland
[d] Department of Radiology, University of Lausanne (UNIL), Switzerland

ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form
31 August 2016
Accepted 23 December 2016
Available online 26 December 2016

ABSTRACT

In the brain, glycogen is a source of glucose not only in emergency situations but also during normal brain activity. Altered brain glycogen metabolism is associated with energetic dysregulation in pathological conditions, such as diabetes or epilepsy. Both in humans and animals, brain glycogen levels have been assessed non-invasively by Carbon-13 Magnetic Resonance Spectroscopy (^{13}C-MRS) *in vivo*. With this approach, glycogen synthesis and degradation may be followed in real time, thereby providing valuable insights into brain glycogen dynamics. However, compared to the liver and muscle, where glycogen is abundant, the sensitivity for detection of brain glycogen by ^{13}C-MRS is inherently low. In this review we focus on strategies used to optimize the sensitivity for ^{13}C-MRS detection of glycogen. Namely, we explore several technical perspectives, such as magnetic field strength, field homogeneity, coil design, decoupling, and localization methods. Furthermore, we also address basic principles underlying the use of ^{13}C-labeled precursors to enhance the detectable glycogen signal, emphasizing specific experimental aspects relevant for obtaining kinetic information on brain glycogen.

1. Introduction

Glucose is a universal energy fuel whose oxidation supports cellular work. The brain, in particular, is highly dependent on glucose oxidation and is, therefore, responsible for a substantial fraction of basal blood glucose uptake [1]. Mammalian cells obtain glucose not only from the blood but also from intracellular stores located in the cytosol. In these stores glucose exists as a polymer: glycogen. Polymerization allows for the presence of large amounts of glucose inside the cell without consequences to osmolality or concentration-gradient based exchanges with the blood, where physiological glucose concentration is ~5 mM. Glycogen is found in several organs, including the brain. In this review we briefly overview the biochemistry, structure and function of glycogen, as elucidated mainly by studies performed in the liver and skeletal muscle, the two largest stores of glycogen in the body. Then, we focus on the role of glycogen in the brain and give some examples of altered glycogen metabolism in human diseases.

Magnetic resonance (MR) methods *in vivo*, notably MR spectroscopy (MRS), are a unique and versatile tool to assess metabolite concentrations in a given organ, and to follow their dynamic changes non-invasively and in real time. Carbon-13 MRS (^{13}C-MRS) is currently the state-of-the-art technique to study glycogen metabolism *in vivo* in humans and rodents. Such studies have explored glycogen dynamics under different physiological conditions and have been expertly discussed in a recent review by Khowaja et al. [2]. Therefore, in the second half of this review, we rather focus on relevant technical and experimental aspects that should be considered for optimization of glycogen detection and data interpretation.

List of abbreviations: ^{1}H-MRS, Proton magnetic resonance spectroscopy; ^{13}C-MRS, Carbon-13 magnetic resonance spectroscopy; B_0, magnetic field; CEST, Chemical exchange saturation transfer; CSI, Chemical shift imaging; FID, Free induction decay; FSW, Fourier series window; FWHM, Full width at half maximum; ISIS, Image selective *in vivo* spectroscopy; MR, magnetic resonance; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NOE, Nuclear Overhauser effect; OVS, Outer volume suppression; RF, Radio-frequency; SAR, Specific absorption rates; SNR, Signal to noise ratio; T_1, Spin-lattice relaxation time; T_2, Spin-spin relaxation time; UDP, Uridine diphosphate; VOI, Volume of interest; γ, Gyromagnetic ratio; $\gamma\Delta B_0$, Field inhomogeneity.

* Corresponding author. EPFL-SB-CIBM-AIT, CH F1 627 Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland.
E-mail address: hongxia.lei@epfl.ch (H. Lei).

http://dx.doi.org/10.1016/j.ab.2016.12.023
0003-2697/

2. Glycogen biochemistry structure and function

Mammalian glycogen is composed of several chains of 11–16 glucose molecules [3] linked by linear α-(1,4) glucosidic bonds or branching α-(1,6) glucosidic bonds [4]. The succession of branches forms a bush-shaped spherical particle (β-particle) organized in tiers, with an average diameter of ~20 nm [5]. This structure is optimized to be stored in the smallest possible volume while keeping the glucosyl units easily accessible to enzymes involved in glycogen metabolism [6]. The glycogen molecule is built from a protein primer, glycogenin [7], and it is now clear that glycogen is directly associated to several other proteins, including those involved in its own metabolism (*e.g.* glycogen synthase, glycogen phosphorylase, or glycogen branching and debranching enzymes) and other metabolically relevant ones [8]. Because these glycogen granules are actually a collective structure of polysaccharide chains with their own metabolic machinery they have been referred to as an organelle-type particle, the so-called glycosome [9].

Glycogen granules assume different structural organizations depending on the tissue of origin and in accordance with the function they serve. For instance, in skeletal muscle, glycogen exists mostly as ~20 nm β-particles, also called proglycogen, whose size may reach up to ~40 nm (macroglycogen) with the addition of more glucosyl units [8]. The abundance of smaller proglycogen particles, with a proportionally higher content of metabolically active enzymes, seems to serve the purpose of ensuring a readily expendable glucose source for muscular work [8]. On the other hand, in the liver and cardiac muscle, β-particles are often clustered into larger aggregates of ~150 nm: α-particles [10]. Such aggregation occurs presumably via covalent or strong non-covalent bonds [11]. Differently from skeletal muscle glycogen, these larger particles with a proportionally lower protein content [9] would be in line with a slower mobilization of glucosyl units for hepatic glucose output during progressive fasting. Interestingly, the formation of α-particles in the liver appears to occur during net glycogen degradation, providing a mechanism to control glucose release [12,13].

3. Brain glycogen

Brain metabolism mainly relies on glucose oxidation. Glycogen represents the largest store of glucose equivalents in the brain [14] with its concentrations ranging from 3 μmol/g[1] to 10 μmol/g in the rodent [15–20] and human brain [21]. Glycogen concentration exhibits regional differences [16,17,22–25], *e.g.* reaching over 20 μmol/g in corpus callosum of rats [17].

Structurally, glycogen exists both as β- and α-particles in astrocytes [26]. Despite expressing all the necessary enzymes for glycogen synthesis, this pathway remains mostly inactive in neurons [27]. At the cellular level, glycogen biogenesis from glucose requires the sequential activities of hexokinase (glucose → glucose-6-phosphate), phosphoglucomutase (glucose-6-phosphate → glucose-1-phosphate), uridine diphosphate (UDP)-glucose pyrophosphorylase (glucose-1-phosphate → UDP-glucose) and glycogen synthase, which adds glucosyl residues from UDP-glucose to the glycogen molecule via α-(1,4) bonds. Branching of the glycogen molecule is afforded by the branching enzyme that catalyzes the formation of α-(1,6) glucosidic bonds. By glycogen phosphorylase activity, glucosyl units are released from glycogen in the form of glucose-1-phosphate, and this intermediate is further converted to glucose-6-phosphate by phosphoglucomutase activity.

While it is generally accepted that brain glycogen is not a first-line oxidative fuel, it becomes metabolically relevant under conditions where energy demand can no longer be met by blood-derived glucose. Indeed, a large body of pre-clinical studies demonstrates such glycogen-dependent neuronal activity in stress situations. Illustrative examples comprise the reduction of brain glycogen levels during intense exercise [28], hypoglycemia [29,30], global ischemia [22] or sleep deprivation [17]. Importantly, it is now recognized that glycogen is key for normal brain function beyond representing an emergency energy reserve [16]. For example, glycogen has been proposed as an expeditious energy source supporting glutamatergic neurotransmission [31]. In agreement, brain glycogen was shown to be of particular relevance during brain activation [32,33] and learning [34–36], when increasing energy demands need to be rapidly met. Conversely, cerebral glycogen stores have to be adequately replenished when neuronal activity is low and a glucose surplus is available, what has been shown to occur during anesthesia, sleeping or hibernation [16].

Therefore, glycogen metabolism in the brain is a highly dynamic process that adapts to activation and resting periods in a complementary fashion. For instance, brain glycogen levels may double relative to the basal when recovering from severe hypoglycemia [29,37], intensive exercise [28], and sleep deprivation [17,38]. This phenomenon closely resembles glycogen supercompensation as described in the skeletal muscle after exercise [39], suggesting a similar organization of glycogen and associated enzymatic machinery in both tissues. Indeed, the muscle isoforms of glycogen synthase and phosphorylase (which are mostly found but not restricted to the skeletal muscle) are also expressed in the brain [40].

The metabolic pathways downstream glycogen degradation that are involved in supporting brain energy metabolism remain to be fully characterized. Although efforts have been made to understand the precise fate of mobilized glycogen carbons, this topic is still subject of debate. Glycogen-derived glucose-6-phosphate may follow one of two pathways: (i) the pentose phosphates pathway or (ii) glycolysis yielding pyruvate that may be further converted to lactate. In particular, the efflux of glycogen-derived lactate from astrocytes has been associated with brain activation, notably in the scope of memory formation [32,36]. It has also been proposed that lactate may serve as a relevant energy fuel for neurons when energy demands increase [36,41]. However, this so-called lactate shuttle hypothesis [42] would only explain a small fraction of neuronal energy metabolism in the activated brain, with most of the glycogen-derived lactate being released from the brain [32].

4. Brain glycogen and energetic dysfunction in disease

Perturbations of glycogen dynamics may account for brain metabolic dysregulation, compromising normal brain function. Studies in genetically modified mice clearly highlight essential roles of glycogen in the brain: for example, mice lacking glycogen synthase in the brain do not have cerebral glycogen and show hampered performance in memory tasks [34] as well as a higher susceptibility to epilepsy [43]. On the other hand, accumulation of glycogen in neurons may contribute to neurological decline with aging, reproducing features of neurodegenerative diseases [44,45]. In addition, defects in glycogen branching leading to the formation of aggregates inside neurons underlie the most severe form of adolescence-onset epilepsy, Lafora disease [45]. Less prominent alterations of brain glycogen metabolism also contribute to brain energy dysfunction associated with other human diseases, such as epilepsy [46] or diabetes [47], as further discussed in the following paragraphs.

[1] μmol/g is an abbreviation of μmol per gram of wet weight of tissue. The use of μmol/g for living tissue assumes an organ density of ~1000 g/L, which makes μmol/g equivalent to mmol/kg and mM.