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Extraction of glycogen on mild condition lacks AIG fraction

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Abstract. – OBJECTIVE: Extraction of animal tissues with cold water or perchloric acid yields less glycogen than is obtained with hot-alkaline. Extraction with acid and alkaline gives two fractions, acid soluble (ASG) and insoluble glycogen (AIG). The aim of this work is to examine the hypothesis that not all liver glycogen is extractable by Tris-buffer using current techniques.

MATERIALS AND METHODS: Rat liver was homogenized with Tris-buffer pH 8.3 and extracted for the glycogen fractions, ASG and AIG. The degree of homogenization was changed to remove all glycogen.

RESULTS: The content of glycogen was 47.7 ± 1.2 and 11.6 ± 0.8 mg/g wet liver in the supernatant and pellet of the first extraction respectively. About 24% of total glycogen is lost through the first pellet. Increasing the extent of homogenization from 30 to 180 sec and from 15000 to 20000 rpm followed with 30 sec ultrasonication did not improve the extraction. ASG and AIG constitute about 77% and 23% of the pellet glycogen respectively.

CONCLUSIONS: Extraction with cold Tris-buffer failed to extract glycogen completely. Increasing the extent of homogenization followed with ultrasonication also did not improve the extraction. Thus it is necessary to re-examine the previous findings obtained by extraction with cold Tris-buffer.

Key Words:
ASG, AIG, Extraction, Glycogen, Homogenization, Liver.

Introduction

The early studies[1,2] showed that glycogen of animal tissues could not be extracted completely with cold water or trichloroacetic acid even with several extractions, and a portion remained in the denatured protein fraction. Total glycogen extraction was achieved only if the tissue is digested with hot alkaline[1-3]. Roe et al[4] proposed that cell glycogen is trapped physically, and the difficulty of extraction is a result of inadequate homogenization. They reported that the total glycogen could be extracted completely in cold acid by using a high-speed homogenizer. Other studies[5-7] showed that the insolubility of some glycogen particles in cold acid is a result of the binding to proteins. Whelan et al[7] reported that acid soluble glycogen (ASG) is composed of large glycogen particles with low protein content. Acid insoluble glycogen (AIG) is composed mainly of low molecular weight (MW) particles. The high protein-to-carbohydrate ratio of AIG is responsible for its poor solubility in acid.

Recently, a new era of research has been conducted to study the physiological importance of glycogen fractions[8-12]. Many of these studies have been done by using the homogenization-free protocol[11]. Applying this method, the tissue is extracted once with cold PCA. It has been shown that ASG is not extracted completely and AIG is contaminated with ASG by using this procedure. Hence, there is a marked difference between the results of this protocol and classical homogenization method[12,13]. Gilbert et al[14] disregard the early experiments on the extraction of glycogen from animal tissues and extracted glycogen once with an extraction Tris buffer with pH 8.3. They deduced that glycogen is constructed only with α and β particles by using HPLC and electron microscopy[15]. It seems that incomplete removal of total glycogen from the tissue may mislead the researches and affect the findings. In the present study, we examined the hypothesis that all liver glycogen is not extractable by Tris-buffer.

Materials and Methods

Liver Sampling

The liver was isolated from male rats (200-220 g) anesthetized by diethyl ether and washed rapidly three times with ice cold isotonic saline and preserved at –70°C immediately[16].

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Tissue Digestion and Ethanol Extraction

The liver tissue was extracted for glycogen using the method proposed by Gilbert et al[14,15]. In brief, 150 mg of liver tissue was ground with 2 mL cold Tris-buffer pH 8.3 for a range of periods (30, 60, 120, 180 sec) and centrifuged 20 min at 15000×g at 4°C. The supernatant was collected and extracted with ethanol at a final concentration of 55% and centrifuged 10 min at 1500 ×g.

The pellet was always discarded by Gilbert et al; so, we examined it for residual glycogen. The pellet was re-extracted for a further period with 2 mL cold PCA[13]. The supernatant was extracted with ethanol and analyzed as pellet ASG. The last pellet was digested with 200 µL of 30% KOH in a boiling water bath for 10 min, extracted with ethanol and analyzed as pellet AIG. The final ethanol-extracted pellets were dissolved in 2 mL distilled water and 10 µL was analyzed for each glycogen fraction. Glycogen was measured by the chemical method of phenol-sulfuric acid optimized previously[17].

Results

Some Glycogen is Lost Via the Pellet

According to Gilbert's protocol, the liver tissue is ground with an extraction Tris-buffer, after centrifugation the sample is separated to the supernatant and pellet. The supernatant is collected for any further analysis whereas the pellet is discarded. We hypothesized that some portion of glycogen including all AIG would be lost via the pellet. Figure 1 shows that all glycogen did not extract completely with Tris-buffer grinding at the first extraction. The content of glycogen was 47.7 ± 1.2 and 11.6 ± 0.8 mg/g wet liver in the supernatant and pellet respectively. It is concluded that about 24% of total glycogen is lost through the first pellet.

The effect of Homogenization Extent on Glycogen Extraction

The extent of homogenization was changed to determine if all the glycogen can be removed by the first extraction. The yield of extraction was increased slightly when the duration of grinding was increased from 30 up to 60 sec at 15000 rpm. Further increase in the time of homogenization up to 180 sec had no significant effect on the efficiency of the extraction. We also examined 180 sec grinding followed by 30 sec ultrasonication, but it failed to liberate all glycogen. The extent of homogenization was also increased to 30 sec at 20000 rpm without any progress (results not shown).

Fractionation of Pellet Glycogen to ASG and AIG

The pellet was analyzed for ASG and AIG to determine the quantity of each fraction. Figure 1 shows that ASG and AIG constitute about 77% and 23% of the pellet glycogen respectively. So

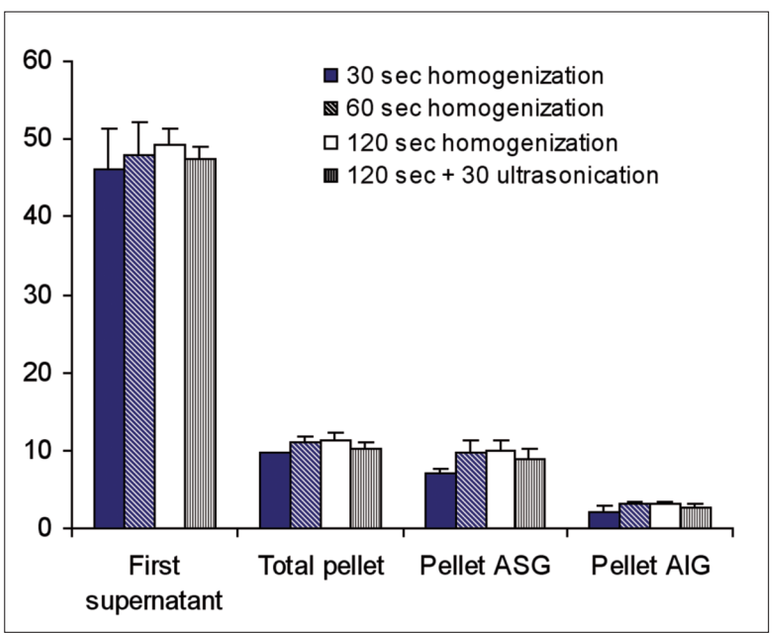

Figure 1. The effect of homogenization extent on the recovery of glycogen fractions. The liver tissue was weighted and homogenized with ice cold Tris-buffer pH 8.3 at different duration and extent and centrifuged 20 min at 15000×g at 4°C. The 'first supernatant' was analyzed as Gilbert's glycogen, i.e. ASG. The pellet was re-extracted with further 2 mL cold perchloric acid and centrifuged, the supernatant was analyzed as 'pellet ASG' and the final pellet was extracted with hot alkaline as 'pellet AIG'. The measurements were done on three samples in duplicate.

AIG is about 0.23 × 0.24 × 100 = 5.5% of total liver glycogen. The results also show that the ratio was not influenced by changing the extent of homogenization.

Discussion

The current study indicates that extraction of the liver with cold Tris-buffer failed to extract glycogen completely, increasing the extent of homogenization followed with ultrasonication also did not improve the efficiency of extraction. The results show that about 24% of total glycogen can be recovered in the first pellet. The pellet glycogen is composed of about 77% of ASG and 23% of AIG. Therefore, AIG is about 5.5% of total glycogen and is found exclusively in the pellet (Figure 1).

The recent reports about the structure of glycogen are based on Gilbert's protocol for the extraction of glycogen from animal tissue[14,15]. The main finding of these works is that glycogen is composed of α and β particles with MWs about 1×10^6 and 100×10^6 D respectively. The glycogen which is obtained by Gilbert's protocol consists of the only ASG, as AIG was discarded by this method. Different results may be achieved if AIG was included in the analysis of glycogen structure.

An accurate analysis of glycogen fractions is also required to study their physiological roles[8-12]. In homogenization-free protocols, the extraction has been done only once by a glass rod followed by unnecessarily high-speed centrifugation[11]. As a consequence ASG is not extracted completely and some extracted ASG precipitates again causing a marked contamination of AIG with ASG. This causes overestimation of AIG, so the homogenization-free procedure has consistently reported more AIG than the researches with homogenization[8-12]. Several extractions with cold PCA are needed to extract ASG quantitatively[12,13]. We also proposed a new method in which total glycogen is divided to the ASG and AIG fractions by adjusting the pH following simultaneous assay[13]. The results of this method are identical to homogenization method.

Conclusions

The extraction with cold Tris-buffer failed to extract glycogen completely, increasing the extent of homogenization followed with ultrasonication also did not improve the extraction. It is suggested to re-evaluate the findings obtained by this method.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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