A Simplified Method for the Isolation and Estimation of Cell Wall Bound Glycogen in *Saccharomyces cerevisiae*

Preetee P. Aklujkar, Santosh N. Sankh and Akalpita U. Arvindekar[1,2]

ABSTRACT

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Glycogen is an important storage reserve in yeast. In *Saccharomyces cerevisiae* glycogen is present in two pools, an intracellular soluble pool and a cell wall bound, insoluble extra-cellular pool. The present method uses a 20% KOH treatment to separate the two pools, which are then estimated using amyloglucosidase. The amount of soluble glycogen was found to be 6.5 mg/g of wet weight of yeast while that of cell wall bound glycogen was found to be almost three times that of the soluble, viz., 18 mg/g of wet weight of yeast. The data is compared with two earlier commonly used methods of yeast carbohydrate fractionation, which reported glycogen in totality. Reviewing these methods in the light of finding two pools of glycogen revealed that both the methods can be demonstrated to yield soluble glycogen in the range of 6–9 mg/g of yeast and 18–21 mg/g of wet weight of yeast of cell wall bound glycogen.

Key words: Cell wall bound glycogen, glycogen estimation, *Saccharomyces cerevisiae*, soluble glycogen.

INTRODUCTION

Glycogen in *Saccharomyces cerevisiae* serves as an important energy reservoir that is correlated to fermentation performance and to the viability of yeast on storage[11]. The concentration of glycogen in yeast varies with changes in environmental conditions[6]. The genes encoding the enzymes of glycogen and trehalose metabolism are induced in similar fashion and to a comparable extent in response to stress, hence it appears that glycogen is probably helpful in combating stress[7]. Glycogen in yeast is present in two pools: one soluble and intracellular while the other is cell wall bound and thus rendered insoluble[4]. The cell wall bound glycogen is linked to the β 1-3 glucan, through a β 1-6 linkage, rendering it insoluble[1]. Further we have found that it is the cell wall bound glycogen pool that relates to ethanol production and fermentation performance[10]. However, the earlier used methods for glycogen estimation have reported glycogen content in totality, in spite of having needed an acid extraction after an alkali extraction to isolate all the glycogen[3,13,14]. (Glycogen can also be estimated using iodine reagent[5] as well as a rapid visualization of glycogen content using iodine has been suggested to determine the yeast status for pitching[9]). Other methods of glycogen estimation involve the physical disintegration of the cell wall and estimation of the glycogen content enzymatically, which also reports glycogen in totality[12].

In the present work, our method of glycogen estimation is compared with the two earlier well-known methods of yeast carbohydrate fractionation, viz. the Trevelyan Harrison method and Manners method[3,13,14]. Several laboratories estimate glycogen content using the Trevelyan Harrison method even today. The present report is intended to put glycogen isolation and estimation in its proper perspective and to demonstrate that the earlier used methods of glycogen isolation and estimation can reveal the presence of two pools of glycogen, which may be serving different physiological functions in the yeast and hence need to be estimated separately.

MATERIALS AND METHODS

Yeast strain

Saccharomyces cerevisiae (NCIM 3494) is a standard laboratory strain, purchased from National Chemical Laboratory, Pune, India. Amyloglucosidase from *Aspergillus niger* was purchased from Sigma Chemical Company, USA. All other reagents were purchased locally and were of analytical grade. The glucose oxidase-peroxidase kit was purchased from Qualigen, India.

Culture conditions

The yeast were routinely grown in 8% glucose, 0.5% peptone and 0.3% yeast extract, at room temperature (25°C) with occasional shaking in 2 L conical flasks containing 1 L media, closed using cotton wool. Cells were harvested after 48 h of fermentation in the stationary phase. About 10–12 g of yeast (wet weight) was collected from 1 L of media.

Isolation and estimation of glycogen

For comparison of the Trevelyan Harrison method and the Manners method, cells were harvested at the end of 48 h of fermentation and washed in ice-cold water. The protocols for fractionation were used according to the original papers, however estimation of glycogen was done

[1] Department of Biochemistry, Shivaji University, Kolhapur, India 416 004.
[2] Corresponding author. Email: drauarvindekar@yahoo.co.in

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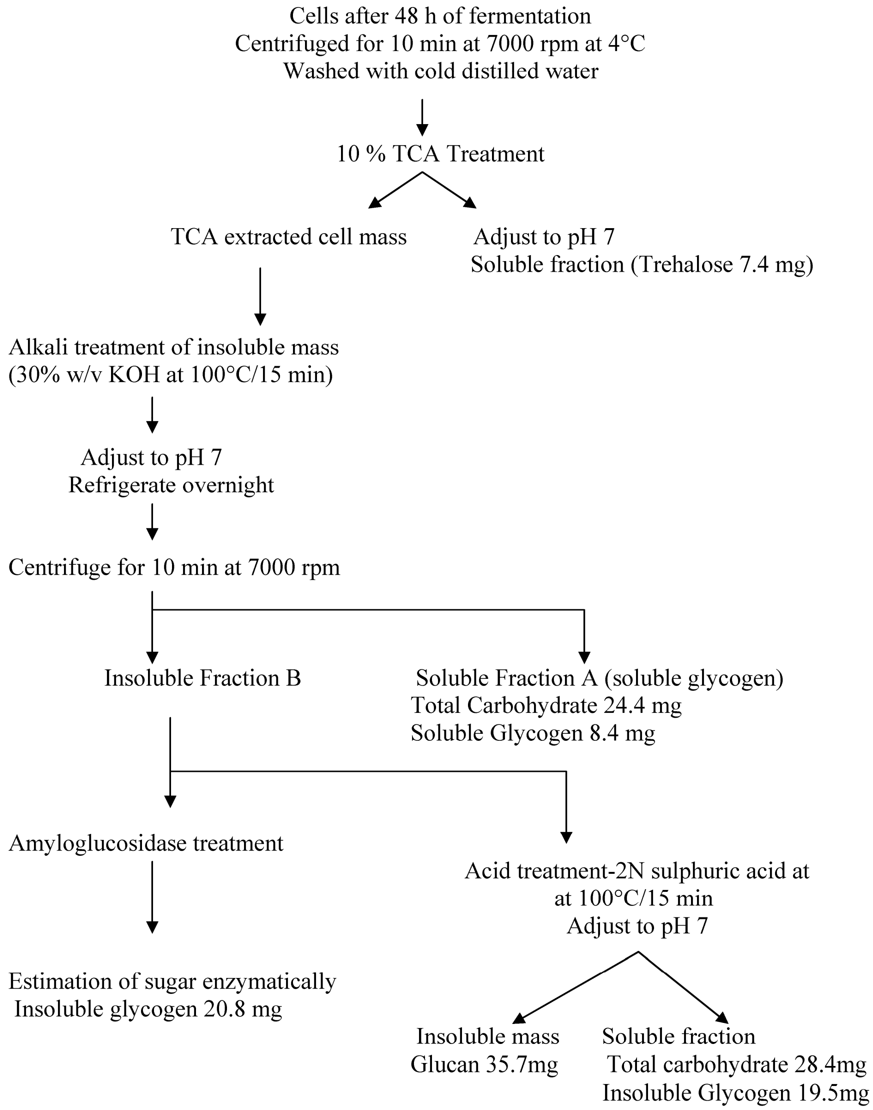

Fig 1. Isolation of soluble intracellular, and insoluble cell wall bound glycogen by the Trevelyan Harrison method. The released glycogen fractions were measured by the glucose oxidase-peroxidase method.

enzymatically using amyloglucosidase and free glucose was measured by the glucose oxidase method. The isolation and estimation of soluble and insoluble glycogen by alkali digestion was carried out as follows. Cells harvested after 48 h of fermentation were washed with ice cold water and 2 mL of 20% KOH was added per g of wet weight of yeast and digested at 100°C for 1 h. The digest was carefully adjusted to pH 7 under cold conditions using 0.2N HCl and the insoluble gel like mass was separated by centrifugation. This was washed repeatedly and subjected to amyloglucosidase treatment for estimating the cell wall bound glycogen. The supernatant was treated similarly to estimate the soluble glycogen fraction. The gel like mass was suspended in water to a volume of 10 mL. Of this, 1 mL was treated with 0.5 mg of amyloglucosidase from *A. niger* in 0.2 M acetate buffer at pH 4.2 for 30 min at 37°C in a total volume of 3 mL. The glucose liberated was estimated by the glucose oxidase-peroxidase method using a commercial kit.

RESULTS AND DISCUSSION

The protocols used for the Trevelyan Harrison method, Manners's method and the method used by us are outlined in Figs. 1, 2 and 3 respectively. With the Trevelyan and Harrison method, the soluble fraction A after alkali treatment and further analysis with amyloglucosidase, showed the presence of an alkali soluble intracellular fraction of glycogen. While the insoluble fraction B requires a treatment with acid to solubilize and release the acid soluble or cell wall bound glycogen. The insoluble fraction B can be treated directly with amyloglucosidase to demonstrate the cell wall bound glycogen or treated with acid, as in case of the original paper to show a similar release of cell wall bound glycogen.

Manners's method, on the other hand, was developed essentially for assessment of β 1-3 and β 1-6 glucan of the cell wall. They reported repeated use of amylase treatment to remove traces of glycogen and still found maltose is

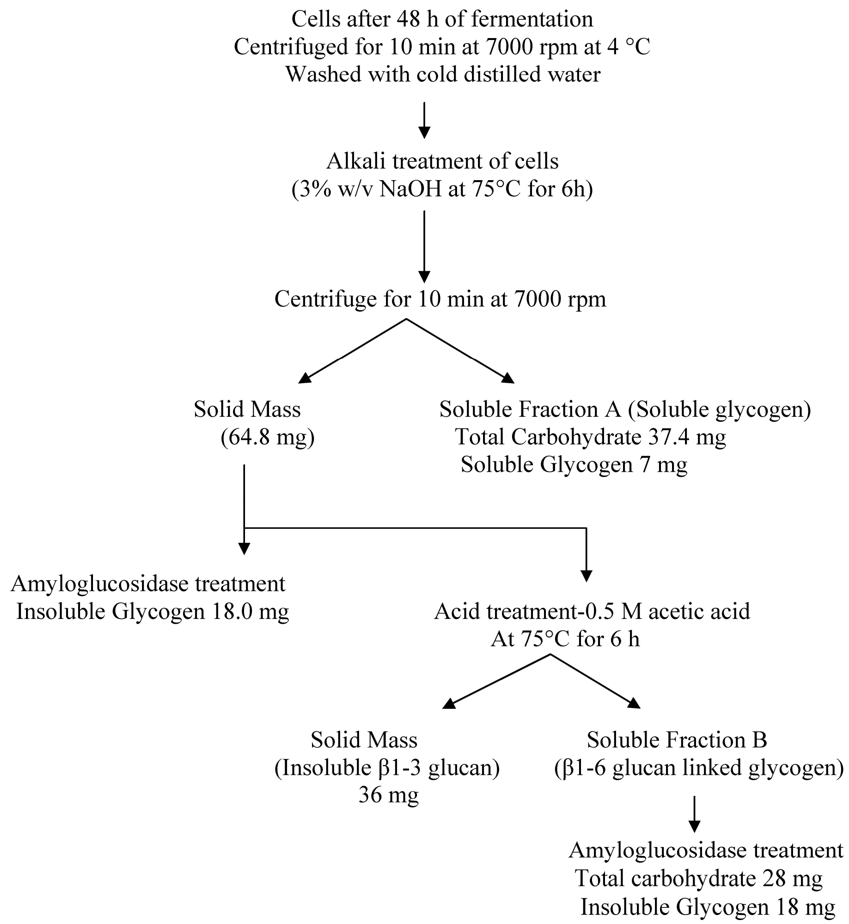

Fig 2. Isolation of soluble intracellular and insoluble cell wall bound glycogen by the Manners method. The released glycogen fractions were measured by the glucose oxidase-peroxidase method.

associated with the beta 1-6 fraction[3]. In Fig. 2 the alkali soluble fraction A contains the soluble glycogen, while the acetic acid treated fraction B shows the cell wall bound glycogen fraction. As in case of the Trevelyan Harrison method, Manners's method with a direct treatment of the solid insoluble mass with amyloglucosidase, yields the same content of cell wall bound glycogen, as after acetic acid treatment.

The method described in Fig. 3 is routinely used in the author's laboratory to estimate soluble and insoluble glycogen. The cells are digested by alkali treatment followed by amyloglucosidase treatment. The ratio of insoluble to soluble glycogen in the three methods is 2.32, 2.57 and 2.77 respectively. The variation in insoluble glycogen content is less (±1.5 mg/g yeast), while it is found to be higher for soluble glycogen (±2.5 mg/g yeast). It is the author's personal observation that on refrigerating the soluble fraction of glycogen overnight, some insoluble aggregates are formed which may be due to contamination of the soluble fraction of glycogen with the cell wall bound glycogen fraction and thus one is likely to overestimate this glycogen fraction.

Although various methods of glycogen estimation were mentioned earlier, only the Trevelyan Harrison method and Manners's method, which involve stepwise yeast cell carbohydrate fractionation have been reviewed in this work. This is in view of the fact that the other methods involve a step of cell disintegration, followed by enzymatic analysis, that estimate glycogen in totality.

It is clear that the glycogen in yeast is present in two pools and this can be demonstrated by the earlier methods of isolation. It was simply assumed that there was a need for alkali and acid treatment to extract glycogen and the values were reported as total glycogen. However, there has been no enquiry into the fact that since glycogen is alkali soluble, why was acid extraction essential. Further, it should be borne in mind that in repeated experiments, the same quantities of alkali soluble and acid soluble glycogen were obtained by all three methods, indicating that these were not artifacts, but separate entities. It has been observed using freeze substitution electron microscopy and specific staining of polysaccharides that glycogen is present as a ring in the cell wall and is well stained in cytoplasm invaginations in the periplasmic space[2].

The insoluble cell wall bound glycogen fraction relates to fermentation performance[10]. It has also been shown that the acid soluble glycogen pool plays an important role as a reserve carbohydrate to supply energy during membrane lipid synthesis in the early aerobic period of fermentation[8]. It would be vital for a brewer to ensure that there is proper repletion of the cell wall bound glycogen prior to pitching. It is therefore important to recognize that glyco-

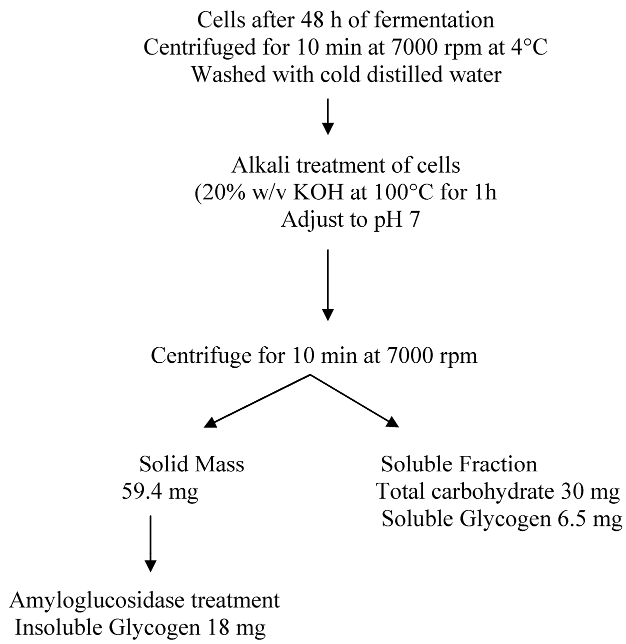

Fig 3. Isolation of soluble intracellular and insoluble cell wall bound glycogen by the alkali digestion method. The released glycogen fractions were measured by the glucose oxidase-peroxidase method.

gen in yeast does exist in two pools, the physiological function of which may differ and thus there is a need for these to be isolated and estimated separately.

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