Carbohydrate Polymers 115 (2015) 305–316

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Rice starch granule amylolysis – Differentiating effects of particle size, morphology, thermal properties and crystalline polymorph

Sushil Dhital[a], Vito M. Butardo Jr.[b,1], Stephen A. Jobling[b], Michael J. Gidley[a,*]

[a] Centre for Nutrition and Food Sciences, ARC Centre of Excellence in Plant Cell Walls, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD 4072, Australia
[b] Plant Industry and Food Futures Flagship, Commonwealth Scientific and Industrial Research Organisation (CSIRO), P.O. Box 1600, Canberra, ACT 2601, Australia

ARTICLE INFO

Article history:
Received 21 July 2014
Received in revised form 12 August 2014
Accepted 13 August 2014
Available online 2 September 2014

Keywords:
Amylopectin
Amylose
Amylose Extender
Digestibility
High amylose
Starch structure

ABSTRACT

The underlying mechanism of amylolysis of rice starch granules was investigated using isolated starch granules from wild-type, as well as *SBEIIb* mutant and down-regulated lines. Fused granule agglomerates isolated from mutant and transgenic lines were hydrolysed at similar rates by amylases, and had similar crystalline patterns and thermal properties as individual granules. Surface pores, a feature previously only reported for A-polymorphic starch granules, were also observed in B- and C-polymorphic rice starch granules. Although the microscopic patterns of hydrolysis among granules with different crystalline polymorphs were qualitatively similar, the extent and the rate of amylolysis were different, suggesting that B-type crystalline polymorphs are intrinsically more resistant to enzymatic hydrolysis than A-type in rice starch granules. It is proposed that the slightly longer branch lengths of amylopectin which leads to the formation of more stable B-type double helical structures compared to their A-type counterparts is the major parameter, with other factors such as granule size, surface pores and interior channels having secondary roles, in determining the rate of enzymatic hydrolysis of rice starch granules.

1. Introduction

The rate and extent of enzymatic hydrolysis of starch has attracted much attention not only due to its central role in converting starch to glucose as the major source of energy in human and animal diets, but also as an important biological (e.g. germination of grains and sprouting of tubers) and industrial process (e.g. syrups and bio-ethanol production). The action of amylolytic enzymes on starch granules may be affected by various factors including granule morphology (e.g. shape and size, presence of compound granules), surface features (e.g. surface damage, pores leading to interior channels, and other as yet incompletely characterised zones of enzyme susceptibility), presence of non-starch components (e.g. proteins, lipids and cell wall remnants), and molecular composition and conformation (e.g. size and amount of amylose and amylopectin, type and amount of crystallinity) as described elsewhere (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009; Colonna, Leloup, & Buleon, 1992; Gallant, Bouchet, Buleon, & Perez, 1992). However, the relative importance of these factors has been difficult to determine as they are typically correlated with each other, and there is a lack of systems available for which individual factors vary whilst other factors are kept constant.

Starch has a broad array of granule sizes ranging from ca. 1 μm (e.g. rice, amaranth, and quinoa starch) to more than 100 μm (e.g. potato and canna starch). Smaller granules, either from different botanical origins (Fukai, Takaki, & Kobayashi, 1994; Ring, Gee, Whittam, Orford, & Johnson, 1988) or fractionated from the same origin (Dhital, Shrestha, & Gidley, 2010; Franco & Ciacco, 1992; Franco, Ciacco, & Tavares, 1998; Noda et al., 2005; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001), are found to be more susceptible to amylolysis than their larger counterparts, consistent with the relatively higher surface area per unit mass available for enzyme adsorption (Warren, Royall, Gaisford, Butterworth, & Ellis, 2011). Cracks, holes or surface damage in granules can further increase the effective surface area enhancing the rate of enzymatic adsorption and binding to macromolecules. Cereal starches such as maize and sorghum are known to have naturally occurring surface pores and interior channels (Fannon, Hauber, & BeMiller,

* Corresponding author. Tel.: +61 7 33652145; fax: +61 7 33651177.
E-mail addresses: s.dhital@uq.edu.au (S. Dhital), v.butardo@irri.org (V.M. Butardo Jr.), steve.jobling@csiro.au (S.A. Jobling), m.gidley@uq.edu.au, mike.gidley@uq.edu.au (M.J. Gidley).
[1] Present address: International Rice Research Institute, Los Baños 4031, Philippines.

http://dx.doi.org/10.1016/j.carbpol.2014.08.091
0144-8617/

1992; Fannon, Shull, & BeMiller, 1993) that may facilitate the enzymes inside the granules affecting the hydrolysis rate and pattern compared to tuber and high amylose maize starches (HAMS), which apparently lack such structural features. Due to the easy access of enzymes towards the less organised granule interior, cereal starches are hydrolyzed at a faster rate from the inside to the outer periphery regions in an 'inside-out' pattern, whereas potato and HAMS granules are hydrolyzed more slowly by exo-corrosion, starting from the surface towards the inside of granules, an 'outside-in' pattern (Gallant, Bouchet, & Baldwin, 1997; Gallant et al., 1992; Zhang, Ao, & Hamaker, 2006). Indeed, the larger "A" granules from cereals such as wheat and barley have clear inbuilt zones of susceptibility to amylolysis around the equatorial groove which facilitates enzyme access for 'inside-out' digestion.

At shorter length scales, the distribution of semi-crystalline and crystalline layers based on double helices formed from amylopectin branches and molecular interaction within and between these layers may also affect the rate and extent of enzymatic hydrolysis. Double helices derived from shorter amylopectin branches (e.g. DP 10–12) form A-type crystallites as found in many cereal starches which may be more readily digestible than the B type crystallites containing longer double helices that are typical of tuber and high amylose cereal starches (Jane, Wong, & McPherson, 1997; Zhang, Venkatachalam, & Hamaker, 2006). However, the presence of surface pores and internal channels in many cereal starch granules, but not tuber and HAMS granules, may also be the rate-determining factor.

Thus, enzymatic hydrolysis of starch granules may be controlled by either the granular structure and/or the organisation of crystallites within the granules. These two factors however have not been investigated separately. Comparative study of potato and HAMS versus cereal starches such as maize or sorghum necessarily compares not only A-type vs. B-type polymorphs but also large differences in either amylose content (HAMS) and/or amylopectin branch lengths (potato) compared with most cereal starches. Indeed, differences in enzyme digestion between maize starches (waxy, normal and HAMS, with amylose contents from almost zero to more than 50%) (Evans & Thompson, 2004; Gerard, Colonna, Buleon, & Planchot, 2001; Morita, Ito, Brown, Ando, & Kiriyama, 2007), although representing the same botanical source, are difficult to assign a structural origin for as there are correlated sets of differences in terms of granular structure and molecular organisation. Furthermore, the study of enzymatic hydrolysis using crystalline substrates such as lintners (crystalline residues of starch after long mild acid hydrolysis) or amylose spherocrystals (e.g. crystallised de-branched amylopectin chains) may not be representative of the lamellar structure of native starch granules and results are often discordant. As an example, Planchot, Colonna, and Buleon (1997) reported A-type spherocrystals to be more readily hydrolyzed compared to B-type spherocrystals, whereas recently Cai and Shi (2013, 2014) showed the opposite effects for a different source of spherocrystals and crystalline aggregates. Thus, the underlying mechanism and factors contributing towards the enzymatic hydrolysis of starch granules are not fully understood.

In this paper, we report the effect of particle size and crystalline polymorph on the amylolysis of a series of related rice starches isolated from wild-type lines compared with mutant and transgenic lines where starch branching enzyme IIb (SBEIIb) is either absent or down-regulated. We previously described the structural, physico-chemical and functional impacts of down-regulating the levels of SBEIIb in rice using artificial microRNA (ami-RNA) and hairpin RNA (hp-RNA) RNA silencing techniques (Butardo et al., 2011). Rice altered by the ami-RNA technique (ami-BEIIb) produced a different starch phenotype to that modified using the hp-RNA technique (hp-BEIIb), with a slightly greater increase in the proportion of long amylopectin and intermediate chains. This resulted in a transition from the normal A-type starch crystalline pattern of the wild type Nipponbare to the C-type for hp-BEIIb and to the B-type for ami-BEIIb. This gives an opportunity to study the amylolysis of starches from cultivars of a single species (*Oryza sativa Japonica* ssp. Nipponbare cultivar) with similar amylose content but with different crystalline polymorphs, to elucidate the underlying mechanism and factors contributing towards the enzymatic hydrolysis of granular rice starches. We also compare the rate of enzymatic hydrolysis for *Indica* rice ssp. wild-type (IR36) and its corresponding *amylose extender* (IR36ae) mutant which was previously shown to have elevations in true amylose, as well as having significantly elevated proportions of long chain amylopectin and intermediate chains (Butardo et al., 2012)

2. Materials and methods

2.1. Materials

Two wild type rice lines were used as reference controls in this study, one in a *Japonica* background (Nipponbare, NIP) and one in an *Indica* background (IR36). They were compared with hp-BEIIb and ami-BEIIb transgenic lines with down-regulated *SBEIIb* generated by *Agrobacterium* transformation of the *Japonica* parent line NIP using hairpin RNA (hp-RNA) and artificial microRNA (ami-RNA) RNA silencing, respectively. We also used IR36ae, an *SBEIIb* mutant of the *Indica* parent line IR36 to serve as a reference control for high amylose rice *amylose extender* mutation.

All the transgenic Nipponbare rice lines used in this study were previously developed in CSIRO Plant Industry (CPI), Canberra, Australia (Butardo et al., 2011). Nipponbare, IR36 and IR36ae were routinely grown in CPI, from the seed stocks originally obtained from Yanco Agricultural Institute, Yanco, NSW, Australia. All the rice lines were simultaneously grown inside a biosafety glass house for this experiment as previously described (Butardo et al., 2011). Mature panicles were harvested, dried, threshed and dehulled, after which the seeds were polished and milled to generate the rice flours used in this study.

2.2. Methods

2.2.1. Isolation and fractionation by sedimentation

Starch was isolated from various rice lines following alkali extraction of the protein. 15 g of milled rice was steeped in 6 volumes of 0.165% (w/v) sodium hydroxide solution (Cardoso, Putaux, Samios, & da Silveira, 2007) at room temperature (ca. 22 °C) for 24 h to soften the endosperms. The steep liquor was drained off and the endosperms were ground lightly into successively smaller fractions using a mortar and pestle. The slurry was then diluted to the original volume with 0.165% (w/v) sodium hydroxide, stirred for 10 min and allowed to settle overnight. The cloudy supernatant (70 mL) was drained off, and the sediment was again diluted to the original volume with 0.165% sodium hydroxide solution. The mixture was then shaken for 10 min and centrifuged at 1250 rpm for 5 min. The process was repeated until the supernatant became clear and gave a negative reaction to the Biuret test for protein. The slurry of starch was suspended in distilled water, passed through a 250 μm sieve and centrifuged at 1200 rpm for 5 min. The washing process was repeated until no pink colour was observed in the presence of phenolphthalein, and finally starches were washed with ethanol and dried overnight at 40 °C under vacuum.

The isolated granules were separated into very small (VS, <4 μm diameter (Ø)), small (S, 4–6 μm Ø), medium (M, 6–8 μm Ø) and large (L, >8 μm Ø) fractions by a repeated sedimentation process in water containing 0.02% sodium azide (Dhital et al., 2010b). The relative yield and median diameter of separated fractions measured