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0144-8617(95)001489-4

Carbohydrate Polymers, Vol. 29, No. 1, pp. 41–44, 1996

0144-8617/96 $15.00 + .00

Enzyme debranching studies on green gram (*Phaseolus aureus*) starch fractions

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(Received 1 November 1995)

The molecular architecture of green gram starch has been studied after fractionation by enzymic methods and size exclusion chromatography. Green gram starch has a high content (ca 30%) of sparsely branched amylose of a relatively high molecular weight (4.5×10^6), highly branched amylopectin, and moderately branched intermediate fraction. The low *in vitro* digestibility with glucoamylase of green gram starch is probably related to its molecular structure. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Starch is the principal dietary carbohydrate (65–78% in cereals and 50–60% in legumes) of humans. Depending upon the source, the isolated starch exhibits varied physico-chemical, functionality and digestibility characteristics. From earlier studies, it is known that the digestibility of legume starches, both in terms of absolute amount digested and also the rate at which it is hydrolysed, is lower (~45%) than that of cereal starches (> 80%), whether in the native or gelatinized form; and that high consumption of legume-based foods leads to flatulence and other physiological discomforts (Tharanathan *et al.*, 1987). The various factors which affect starch digestion in cereal- and legume-based diets are the amylose content, starch–protein and starch–lipid interactions, extent of gelatinization and retrogradation and the presence of any antinutritional substances such as enzyme inhibitors, phytic acid, phenolic compounds, lectins, etc. (Gupta, 1987). It is known that the higher the amylose content, the lower the starch digestibility (Ueda *et al.*, 1974). Most commonly, the amylose content of cereal starches (~15–25%) is less than that of legume starches (~35–42%) and this has been shown to play, at least partly, a significant role in the starch digestibility characteristics (Glennie, 1987). The overall differences in the *in vitro* digestibility values are primarily attributable to the nature and composition of starch *per se*. The present investigation is an attempt to look into the molecular details of green gram (*Phaseolus aureus*) starch with regards to its digestibility.

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EXPERIMENTAL

Green gram was purchased in the local market. After cleaning, the seeds were dehusked by milling and powdered to 170 mesh size. The resulting flour was steeped in water for 12 h, sieved through 240 mesh and the crude starch thus obtained was suspended in dilute NaOH (pH 9.0) for 10 min followed by centrifugation and water washings. The starch was further purified by repeated washings with 0.1 M NaCl–toluene (10:1) and finally water washed and dried by alcohol and ether washings (Madhusudhan *et al.*, 1993). Fractionation was done by 1-butanol complexation (Banks & Greenwood, 1967) and concanavalin A precipitation (Yun & Matheson, 1990) methods, and the crude amylose was further resolved into pure amylose and an intermediate fraction by the hot 1-butanol extraction method (Takeda *et al.*, 1990). β-Amylolysis (Atwell *et al.*, 1980) and debranching with pullulanase followed by gel permeation chromatography (GPC) were performed according to Biliaderis *et al.* (1981). For size exclusion–high performance liquid chromatography (SE–HPLC), a precalibrated (with dextrans of known molecular weights) Sepharose CL-2B (1.7 × 92 cm) was used (Kobayashi *et al.*, 1985), with water as the eluant. The starch fractions (10 mg/ml) were solubilized in aqueous DMSO (85%) by heating at 95°C for 5 min, centrifuged and the clear supernatants (10 μl) were injected. The various analytical methods were performed as reported elsewhere (Changala Reddy *et al.*, 1990). The relative viscosity, η_r, of starch solutions in 1 M KOH at 0.5% concentration was measured at 25±0.5°C in an Ostwald Viscometer. Limiting viscosity number, η, was determined by extrapolation to zero concentration from a

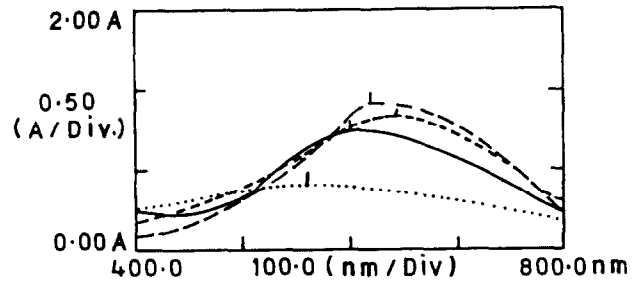

Fig. 1. λ_{max} of starch I_2 blue colour of green gram and its fractions: - - -, starch; ···, Ap; – – –, Am; ——, Ax.

Table 1. Characteristics of Am, Ap and Ax fractions of green gram starch

	Am	Ap	Ax
I_2–KI colour	Deep blue	Purple	Blue
λ_{max} (nm)	642	560	609
Molecular weight (kDa)	4.5×10^6	1.7×10^7	9.1×10^6
β-amylolysis limit (%)	87.5	54.2	62.5
$[\eta]$ (dl/g)	25	40	—
General structure	Sparcely branched	Highly branched	Moderately branched

plot of reduced viscosity (η_{sp}/C) vs concentration (C) (Suzuki *et al.*, 1981).

Gelatinized starch granule suspension (100 mg in sodium acetate buffer, pH 4.8, 0.05 M, 4 ml) was incubated with glucoamylase at 60°C for 30 min. The enzyme was heat inactivated, centrifuged and the supernatant was made up to 15 ml. The liberated glucose was analysed by the glucose oxidase method (Dahlqvist, 1964).

RESULTS AND DISCUSSION

Of the three different fractionation methods employed, concanavalin A gave amylopectin in a pure form (Ap, 61% yield), which upon fractionation on SE–HPLC was eluted as a single peak at 14.32 min. The crude amylose fraction present in the supernatant was further resolved by hot butanol extraction into pure amylose (Am, butanol-insoluble, 29.7%) and an intermediate fraction (Ax, butanol-soluble, 6.8%). Both were found to be homogeneous by GPC on Sepharose CL-2B and SE–HPLC; Am emerged at 22.21 min and Ax at 16.26 min. All the fractions showed a varied λ_{max} in the I_2–KI blue colour (Fig. 1). The λ_{max} of Ax was in between those of Am and Ap (see Table 1).

The β-amylolysis (Table 1) of Am, Ap and Ax with pure β-amylase gave β-limit dextrins (β-LD). Native fractions as well as the derived β-LDs upon pullulanase debranching and GPC on Biogel P-10 gave chain profiles as depicted in Figs 2–4, respectively. It may be inferred that the so-called 'linear' amylose fraction is not linear, instead it has some amount of sparsely distributed short-chain branches. The β-amylolysis limit of Ap (~54%) was very much within the range of other starch amylopectins (~52–56%). The absence of a V_o peak suggests complete debranching of Ap. The elution profile shows Ap to have three types of chains with degree of polymerization (DP) 17, 56 and 70, whereas

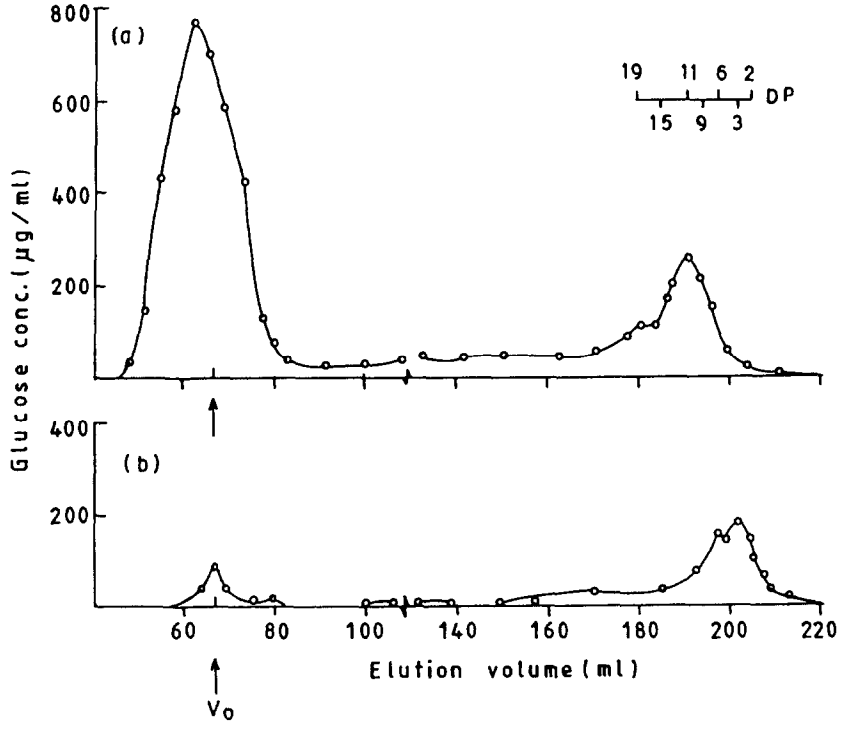

Fig. 2. GPC profile on Biogel P-10 of debranched green gram starch: (a) amylose, and (b) its β-LD.

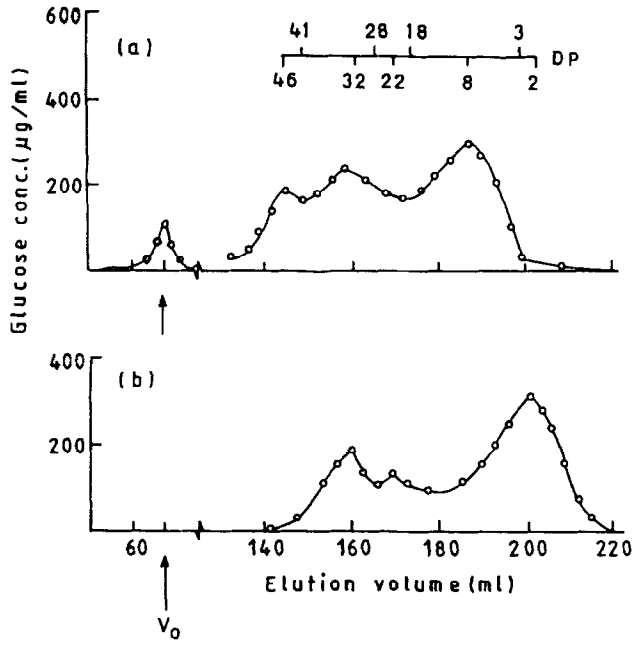

Fig. 3. GPC profile on Biogel P-10 of debranched green gram starch: (a) intermediate fraction, and (b) its β-LD.

its β-LD revealed considerable amounts of maltose and maltotriose in addition to large peaks due to chains of DP 41 and 54 (Fig. 4). These would probably represent the long, medium and short B-chains, whereas chains of DP 17 would constitute normal A-chains. Assuming equal number of A- and B-chains (Manners, 1989), the computed exterior (ECL) and interior (ICL) chain length values were 14.0 and 7.0, respectively, based on an average chain length (CL) value of 22.2 for the Ap of green gram starch.

Contrary to the above, the Am fraction on pullulanase action gave a major V_0 peak together with small amounts of peaks having low DP values (11 and 17; Fig. 2). The former was indicative of a high molecular weight linear molecule resulting from the debranching of Am. On the other hand, debranching of its β-LD gave essentially maltotriose and a peak of DP 6, which represent short A-chain stubs.

The results obtained on the debranching of Ax and its β-LD were of different nature, in that the native fraction gave a number of peaks (DP 8, 32 and 46; Fig. 3), along with a V_0 peak, which could be the amylose impurity, resulting from an incomplete fractionation (most unlikely) or it may represent a truly linear high molecular weight material. The profile of debranched β-LD showed predominantly large amounts of maltose and a few maltooligosaccharides (DP 22 and 32).

The *in vitro* digestibility (Madhusudhan, 1994) of green gram starch was low (~49%) in comparison to

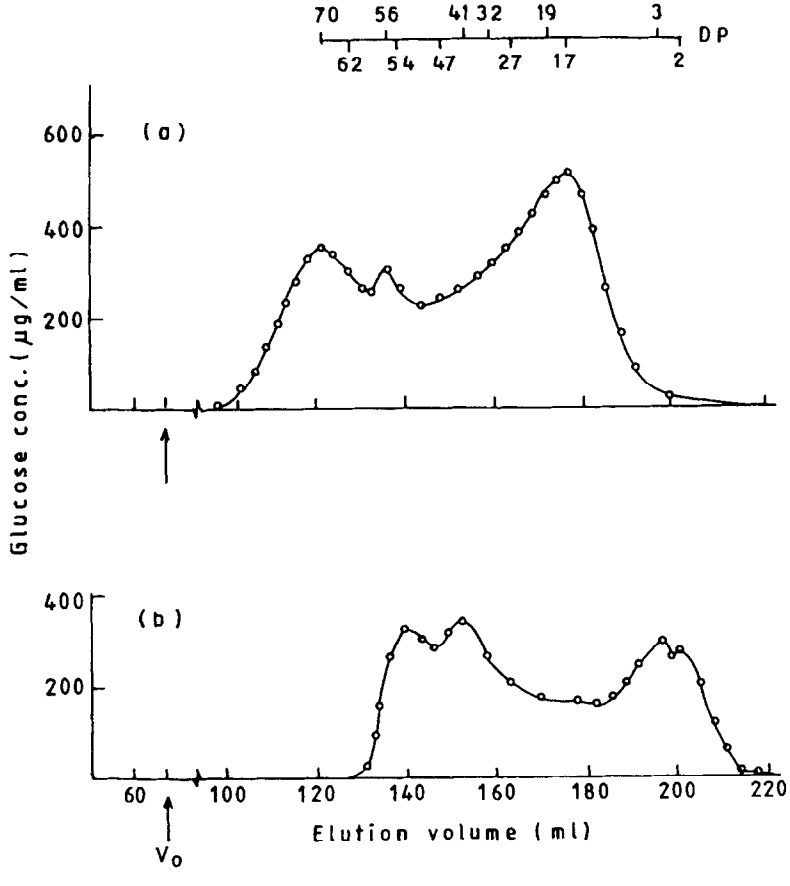

Fig. 4. GPC profile on Biogel P-10 of debranched green gram starch: (a) amylopectin, and (b) its β-LD.

that of rice starch (~88.3%). The former was attributed to some of the properties of isolated starch, more so with regard to its molecular architecture. High amylose content, low solubility and swelling power in water and DMSO, low viscosity values and the presence of protein and lipid in legume starch are shown to affect the starch digestibility value as well as digestibility rate. From the results, an indirect relationship may be invoked, namely, that the presence of a large amount of amylose retards the gelatinization and swelling of starch granules, for reasons that are not clearly understood. It is reported that the solubility of starch granules in DMSO is a measure of susceptibility to amylase action, the higher the solubility the better the amylolytic digestibility (Leach & Schoch, 1962). The branched amylose molecule in green gram starch is yet another contributing factor for its low digestibility.

ACKNOWLEDGEMENTS

BMS thanks the University Grants Commission, New Delhi, for a research fellowship under the FIP scheme. The authors thank Dr (Mrs) Lalitha R. Gowda for help with SE–HPLC analysis.

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