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► To cite this version:

Agnès Rolland-Sabaté, Florent Grimaud, X. Roussel, C. Lancelon-Pin, S. Laguerre, et al.. Structural features of hyperbranched α -glucans obtained in vitro using a new enzymatic cocktail. 26. International Carbohydrate Symposium, Jul 2012, Madrid, Spain. 1 p., 2012. hal-02810621

HAL Id: hal-02810621

<https://hal.inrae.fr/hal-02810621>

Submitted on 6 Jun 2020

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Structural features of hyperbranched α -glucans obtained *in vitro* using a new enzymatic cocktail

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In most living organisms energy is stored in the form of branched α -glucans: starch in green plants, red algae and some cyanobacteria and glycogen in animals, bacteria and fungi. Amylopectin, the main starch component, and glycogen are mainly built of $\alpha(1,4)$ linked glucosyl units with respectively 5-6% and 7-10% of $\alpha(1,6)$ glycosidic bonds corresponding to branching points. Their biosynthesis requires the coordinated action of elongating and branching enzymatic activities, of which the synergetic action is still not clearly understood. Using sucrose as a unique substrate and two bacterial transglucosidases, the amylosucrase from *Neisseria polysaccharea* and the branching enzyme from *Rhodothermus obamensis*, we have developed a biomimetic system to reproduce *in vitro* activities involved in the formation of $\alpha(1,4)$ and $\alpha(1,6)$ glycosidic linkages during starch and glycogen biosynthesis. To get more insight into the synthesis mechanisms and to tailor products with targeted structure, the sucrose concentration and the ratio between amylosucrase activity and branching activity were varied in single step syntheses.

The size and the structure of the α -glucans obtained by the coordinated action of the two enzymes were determined by using complementary enzymatic, chromatographic, scattering, NMR and imaging techniques. In particular, a method using Asymmetrical Flow Field Flow Fractionation was developed especially for in depth analysis

of the structural heterogeneity of these α -glucans by static and dynamic light scattering. New spherical particles with a diameter ranging from 10 to 150 nm, a branching degree ranging from 10 to 13 % and a structure comparable to that of native glycogen were produced. The synthesized α -glucans structural features and synthesis conditions results will be discussed.

Keywords: Hyperbranched α -glucan, glycogen, amylosucrase, branching enzyme, biomimetic system.

The authors gratefully acknowledge funding from Agence Nationale de la Recherche under grant number ANR-09-CP2D-07-01.

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