NMR Identification of Substructural Glucose Units in the Amylopectin Superstructure: The example of Rhodella Violacea Amylopectin

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Abstract

In the course of a study on Rhodella Violacea (Rhodophyta) starch was isolated as the reserve polysaccharide. The two fractions amylose and amylopectin were separated and each fraction was analysed by multidimensional high field NMR spectroscopy. Several glucose residues occupying segregate positions in the amylopectin macromolecules displayed different resonance frequencies. The structural characterisation -proton spin-systems and carbon resonance frequencies- of these atypical glucose sub-units was undertaken. The results of these analysis are presented.

Introduction

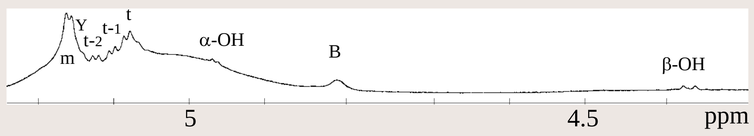
Starch is an ubiquitous reserve polysaccharide form in photosynthetic organisms. Although the basic structural features of the two constituting macromolecular families were recognised very early (linear poly- α(1-4) glucopyranose chains for the amyloses and highly α(1-6) branched oligo- α(1-4) glucopyranose chains for amylopectins), the intricate structural organisation of the branching network of amylopectin at the molecular level still remains poorly known. Unlike proteins, the structures of polysaccharides are not encoded in the genome but are the result of the combined action of enzyme complexes and intrinsic physicochemical characteristics. The various models developed in the past decades to describe the primary structure of amylopectin are still incomplete. Recent models put forward the crucial role of enzymatic chiselling in the biosynthesis of the amylopectin framework [1]. Among the key parameters that remain to uncover are the intra-molecular distances between α(1-6) branching chains. A measurement of these parameters would allow a better understanding of the differences observed between amylopectins of various botanical origins. Unfortunately most methods used in structural analysis can provide little more than average values of these parameters.
High field NMR spectroscopy provides a mean to investigate insights in polysaccharide's substructures. In a previous paper we described how several glucose residues at the non-reducing ends of highly acetylated wheat starch amylopectins can be identified [2]. In the present work we present the results obtained in identifying the proton and carbon NMR chemical shifts of several, glucosyl residues occupying each a specific location on the native amylopectin macromolecule.

[1] S. Ball,,H.P. Guan, M. James, A. Meyers, P. Keeling, G Mouille, A. Buléon, P. Colonna and J. Preiss *Cell*, 86 (1996) 349-352
[2] B. Laignel, C. Bliard, G. Massiot and J.M. Nuzillard, Carbohyd. Res., 298 (1997) 251-260

Rhodella Starch

The purple algea Rhodella Violacea (Rhodophyta) accumulates starch as the reserve polysaccharide during its growth Period. In a set of experiments, the organism was cultivated under two different conditions : nitrogen rich (N+) and nitrogen poor (N-) media. The reserve polysaccharides were extracted from both culture conditions. Amylose and amylopectin from each polysaccharide fractions were fractionated on a Sepharose ® Cl2B GPC column using 10 mM NaOH. The isolated fractions were pooled and analysed by NMR after exchange of OH protons by Deuterium in D_2O[3]. The spectra of amylose from both culture conditions appeared as a very simple α-D-glucopyranose substituted in position –4, due to the chemical environment similarity of all monomer within the polysaccharide.
With its much higher degree of branching, amylopectin fractions displayed much more complex nmr spectra. The very high spectral resolution obtained with the amylopectin fraction from the N+ medium allowed us to identify the chemical shifts and entire spin systems from several glucose monomer occupying specific positions in the macromolecule such as -non reducing end and the neighbouring positions, branching residues, reducing ends etc. The structural characterisation -proton and carbon resonance frequencies- of these anhydro-glucose sub-units was undertaken. The results of these analysis are presented.

[3] A Rahaoui, Thèse de Sciences USTL Université des Sciences et Technologies de Lille Flandres Arthois 06 02 1999.



The 4.3 to 5.2 ppm 1H NMR spectral window displays the well separated anomeric protons resonance frequencies corresponding to the regular (m),branched (Y), terminal and the two neighbouring (t, t-1, t-2), reducing (α-OH and β-OH) as well as the branching (B) anhydro-glucose motives.

NMR spectra

The samples were exchanged in excess D2O, incubated at 80 °C for 2h then freeze dried the operation was repeated twice. The exchanged sample was dissolved in DMSO.
The spectra were recorded in DMSO at 100°C. The DMSO residual signal at 2.5 ppm was used for the calibration of the spectra.

A combination of homo- and hetero-nuclear multidimensional NMR experiments - ^{13}C Jmod, COSY, HMBC, HMQC, as well as relayed COSY (relayed once and twice)- were used in the determination of proton spin systems and carbon resonance frequencies.
It was therefore possible to assign the entire spin systems and ^{13}C chemical shifts of anhydro-glucoses in external positions (non-reducing terminal (t) and reducing (α an β- OH)) two of the neighbouring positions (one before last (t-1) and two before last (t-2)) and as well as two interior positions (branching (B) and branched (Y) glucose residues as represented on fig 1.

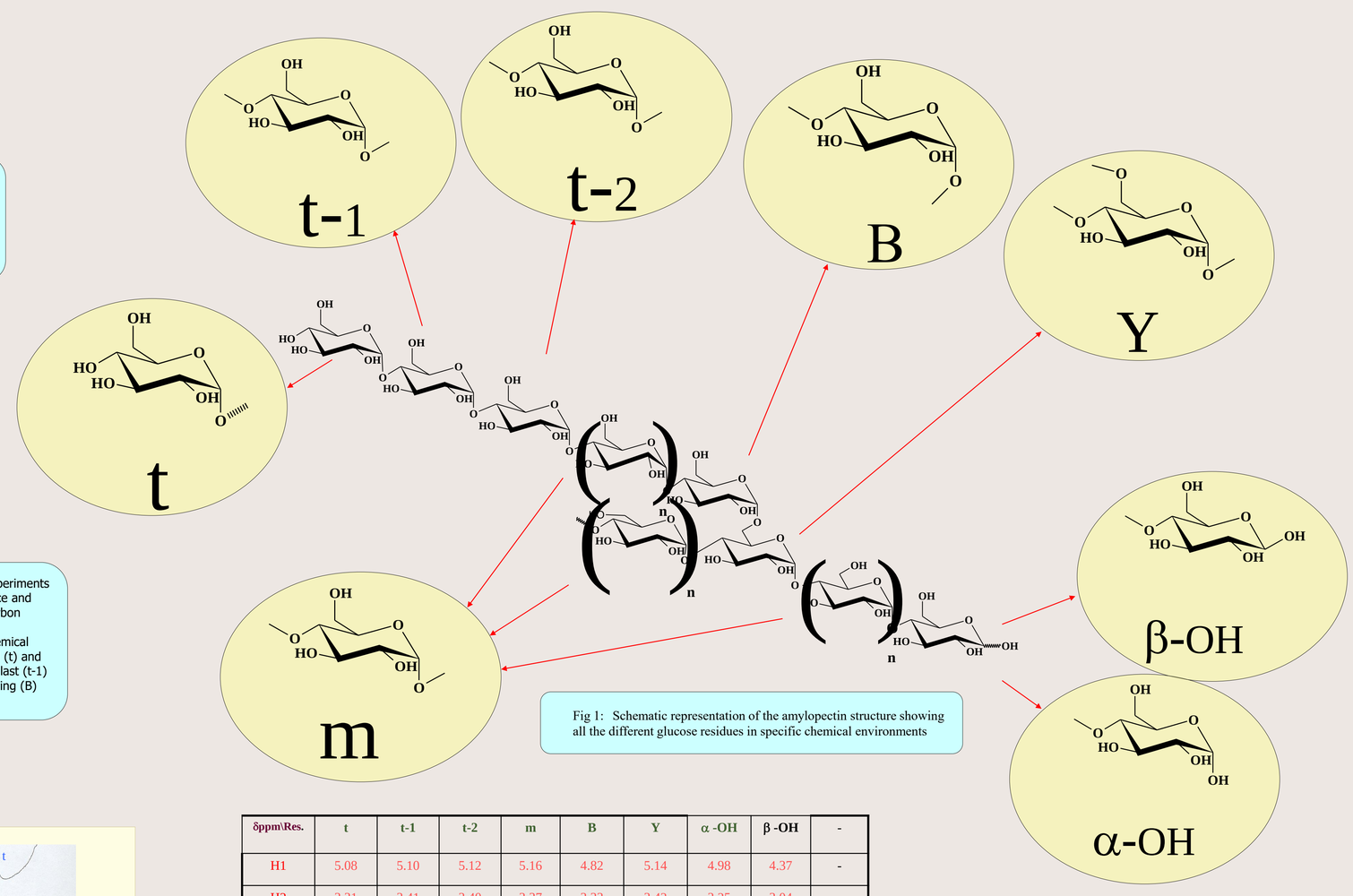


Fig 1: Schematic representation of the amylopectin structure showing all the different glucose residues in specific chemical environments

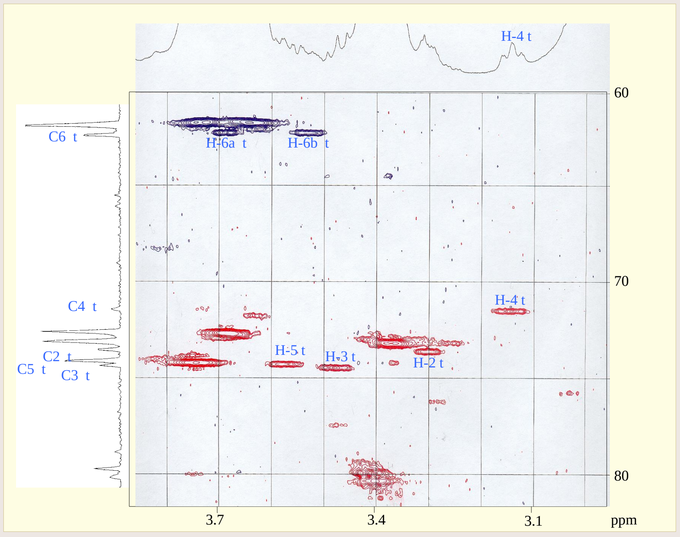


Fig. 2 : Example of phase sensitive hetero-nuclear ^{1}H - ^{13}C direct coupling spectrum used in the determination of glucose residues in specific position on the macromolecule.

δppm\Res.	t	t-1	t-2	m	B	Y	α -OH	β -OH	-
H1	5.08	5.10	5.12	5.16	4.82	5.14	4.98	4.37	-
H2	3.31	3.41	3.40	3.37	3.33	3.42	3.25	3.04	-
H3	3.47	3.74	3.74	3.74	3.82	3.75	3.72	3.47	-
H4	3.15	3.40	3.40	3.41	3.44	3.42	3.39	3.39	-
H5	3.58	3.74	3.74	3.68	3.62	3.90	3.75	3.28	-
H6a	3.69	3.74	3.74	3.76	3.68	3.80	3.75	3.61	-
H6b	3.55	3.74	3.74	3.64	3.62	3.80	3.75	3.76	-
-	-	-	-	-	-	-	-	-	-
C1	101.5	101.3	101.2	101.0	100.0	101.4	93.1	97.9	-
C2	73.6	73.2	73.2	73.2	73.2	73.0	73.3	75.7	-
C3	74.5	74.3	74.3	74.3	74.1	73.9	74.5	77.4	-
C4	71.5	-	80.0	80.0	79.9	80.0	81.0	80.8	-
C5	74.3	-	-	72.6	71.8	71.3	72.4	76.2	-
C6	62.3	-	-	61.5	61.7	68.0	62.5	62.0	-

Tab. 1: ^{1}H and ^{13}C NMR identified chemical shifts of the non- reducing end (t), two neighbouring anhydro-glucose (t-1, t-2), repetitive poly-maltosyl (m), the α 1-6 branching glucose residue (B), the 6-substituted branched residue (Y) and the reducing hemiacetal end (α-OH and β-OH).

Conclusion

The purified amylopectin fraction from the reserve polysaccharide accumulated by the red algae Rhodella Violacea (Rhodophyta) was isolated and analysed by NMR. Eight different anhydro-glucose residues occupying various positions in the polysaccharide and having different resonance frequencies were identified using a series of multi-dimensional homo and heteronuclear NMR experiments.

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