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Note

A high amylose starch isolated from the tubers of *Curcuma aeruginosa*

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NMR spectra of the starch, isolated from *Curcuma aeruginosa* in dimethyl sulphoxide-d_6, contain groups of minor ^{13}C, ^{1}H, or $O^{1}H$ signals attributable to glycosyl end groups and the absence of other residues associated with branching in amylopectin molecules. The relative intensities of these signals are evaluated in terms of the incidence of end groups, or branches in the starches. The α configuration of the glucan has been confirmed by treatment with α amylase, and also by its high $[\alpha]_D^{20}$ value of +118.9.13. ^{13}C NMR and ^{1}H NMR spectral studies show that it is a high amylose starch. X-ray diffraction study reveals B-type polymorphic forms characteristic of high amylose starch. They are also compared with spectrophotometric measurements on the iodine complexing capacity of starches.

Keywords*: Curcuma aeruginosa*, starch, ^{13}C NMR, ^{1}H NMR, X- ray diffraction.

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Starch from *Curcuma aeruginosa*, is used as a substitute for arrowroot. Two main components of starch, amylopectin and amylose have different properties and are thus not suited for the same application[1]. X-ray diffraction studies on amylase fibres[2], demonstrated that both A and B type structures contain ordered arrays of double helices. A-type adopting a nearly close packed arrangements and B-type structure being more open and containing significantly more water between helices[3]. A third C-type is occasionally present in native starches. There is good evidence[3] that the amylopectin component is responsible for the observed long range order through regular packing of double helices formed from adjacent branches. The amylopectin component is responsible for the crystallinity of starch granules. Amylose and amylopectin of starches are readily differentiated[4] by chemical reactions such as iodine binding, methylation analysis, periodate oxidation, and by enzymatic means. Their NMR spectra also differ notably, particularly in high field, ^{13}C NMR spectra of solutions in dimethyl sulphoxide[5]. Information about the amylopectin content is directly accessible from NMR spectra[6], which can distinguish ^{13}C or ^{1}H nuclei of glycosyl end groups and residue engaged in branching from those of main-chain residues in the molecule. Iodine binding measurements provide a ready estimate[7] of the percentage of amylose in a starch. X-ray diffraction can also be used to differentiate high amylose starch and amylopectin starch. Observation on the NMR spectra of starch from *C. aeruginosa*, and its X-ray diffraction results are reported here, and are assessed in the light of data obtained from spectrophotometric measurements on their iodine binding properties.

Starch was isolated from the tubers of *Curcuma aeruginosa* and purified by gel chromatography using Sephadex G 200 column gave a symmetrical elution curve. Its homogeneity was again tested by Zone electrophoresis using borate buffer *p*H 9.3.The MW determined by GPC was found to be 200 KDa. Enzymatic hydrolysis of starch was conducted with α amylase and glucoamylase and the subsequent spectrophotometric analysis. In the IR spectrum the broad peak at 3402 cm^{-1} is due to O-H stretching frequency; the hydroxyl group is engaged in intermolecular and intramolecular H-bonding.

An indication as to the kind of constituent residues present in a polymer, and the relative amounts of these species is readily apparent from the chemical shifts of the signals and their relative intensities in the spectrum integral[8]. In general, the C1 signals for the α glycosidic linkages range between 99-102 ppm. While C-1 signals for the β glycosidic linkages are little down field at 102-105 ppm[9]. For ^{13}C NMR spectrum under study; C-1 signal is at 100 ppm which showed α configuration. High positive optical rotation and its hydrolysis with α amylase confirmed this. ^{13}C NMR spectrum is identical to the one obtained for high amylose starch[7]. ^{13}C NMR spectra of amylopectin in DMSO exhibit a number of resonances that may be ascribed[5,6] to D-glucosyl residues that are associated specifically with branching. The resonance due to anomeric carbons in the glucan was assigned by

virtue of their position to low field[10]. The low field group is assigned to the anomeric carbons involved in (1→4) linkages by comparison with the data for amylose and panose. The C-1 carbons are the only carbons with two directly attached oxygen atoms; thus they are found at lower field than the remaining five carbons. The peak at 100ppm corresponds to C-1 signal involved in α (1→4) linkages. Although branch point residue is the complement of end group its C-6 resonance is barely detectable (at δ 67-68), due to broadening caused by lessened rate of segmental motion of the trisubstituted branching residue within the polymer. This is assigned for C6 involved in α (1→6) linkages[11-13]. Resonance at 100 and 60; by comparing with the observed chemical shift in solution[5] may be assigned to C-1 and C-6 sites respectively. The major signal intensity in the range 68-78 ppm is due to C-2, 3, 4, and 5 sites. Information on the linkage and the anomeric ring carbon was deduced by ^{13}C NMR spectral data. In agreement with literature data[6,14,15], the spectral signs in the region 100, 71-79 and 60.4 ppm were assigned to carbon nuclei C1-C6 of α 1,4-D-glucan. An examination of the spectra shows that the chemical shift of their anomeric carbons is identical (100 ppm). The presence of only one resonance indicates complete anomeric homogeneity[12]. The presence of doublet in the region 100-102 ppm was attributed to C1 of crystalline polymorph[8]. Less crystalline starches are devoid of this doublet (in the present study). The spectra of the polysaccharide from *C. aeruginosa* have the same appearance as that of high amylose starch[7]. The signals at the end unit carbon atoms are noticeably less prominent than in high amylopectin starch or amylopectin. This is not only due to their lower amylopectin content, but also to the fact that resonances of C-1 to C-6 of the amylose present were virtually coincident in chemical shift with the main chain residues of the amylopectin. Hence, a high amylose starch produces a relatively weak group of signals due to the end units.

^{1}H NMR spectra are also similar to that of high amylose starch. The peaks at δ 5.4 and 5.00 are assigned to H-1 adjacent to α (1→4) and α (1→6) linked units and peaks at 5.38 and 4.8 ppm to the α and β forms of the reducing units respectively. The signals at δ 5.4-5.5 in the spectrum shows that signals for OH-2 and OH-3 are strongly deshielded by intra molecular H-bonding involving OH-3 as the principal donor[16]. The prominent signals at δ 5.4-5.5 are also observed in amylopectin, indicates that analogous intramolecular H-bonding also persists in amylopectin. A very small peak at δ 4.9 is observed for the spectrum under study; due to the smaller incidence of (1→6) linkages. Iodine binding measurements gave a high blue value (like high amylose starch), value higher than that of potato starch, another high amylostarch.

X-ray diffraction indicates that starch granules have crystalline properties; however, they are only partly crystalline and vary in their degree of crystallinity. X-ray diffraction pattern showed identical d spacing at 15.79 (medium), a double at 8.63 and 7.72 (very weak), another double at 6.90 and 6.14 (weak), and single spacing at 5.55 (strong), 4.46 (weak), 4.07 (medium), 3.67 (medium), 3.28 (weak) and finally 2.63 (weak)**(Table I)**. The composition of these spacings is characteristic of B pattern, being well recognized for most kinds of tuber starches, in contrast to the A pattern of cereals and C pattern of legumes with other characteristic d spacing[1,17]. The relative abundance of these diffractions and the scattering intensity are essentially dependent on the proportion and length of the branching residues[19-21]. The B pattern is produced by amylomaize starches, which have an amylose content greater than 40-45 %. Most starches consist of about 75 % semi crystalline amylopectin and 25 % essentially amorphous amylose. In the B-type, the double helices are intermitted by water channels resulting in hexagonal units cells[22-25]. A variety of techniques has been used to determine the absolute crystallinity of native starch but the values obtained are very dependent on the technique used.

Table I — X-ray characteristics of *C.aeruginosa*

d- spacing, Å	2θ	Intensity[b]
15.79	5.59	m
8.63	10.25	w-
7.72	11.46	w-
6.90	12.82	w
6.14	14.4	w
5.55	15.93	s
5.12	17.32	s
4.46	19.89	w+
4.07	21.81	m
3.67	24.20	m-
3.28	27.15	w
2.63	34.05	w

[b] Intensity scale; Strong (s); Medium (m); Weak (w); Less than (-); More than (+).

Experimental Section

General methods of analysis. All the chemicals/ reagents were of analytical grade. Glucoamylase (E.C.3.2.1.1), α amylase (E.C.3.2.1.3) were from Sigma Chemical Co; USA. IR spectra were taken with a Hitachi EP1-G3 spectrometer using KBr pellets under dry air at room temperature. NMR spectra were recorded with a Varian XL 300 spectrometer (5mm probe) operated at 75MHz. In the ^{1}H-decoupled mode, a flip angle of 45° was used, and 10,000-40,000 scans were accumulated in a pulsed FT mode. Chemical shifts are referenced with respect to Tetramethylsilane; by use of the solvent DMSO-d_6 signal at δ -39.6. X-ray diffraction patterns were obtained by using a Philips Powder Diffractometer (PCW 1050/1390) mounted on a PW 1730/10 sealed tube X-ray generator operting at the Cu-Kα - wavelength (1.542Å).The relative intensity was recorded in the scattering range(2θ) of 4-40°. The isolation and purification of starch followed general method[26-29].The molecular weight was determined by gel chromatography using standard dextran as marker[27]. Total α glucan was estimated by using enzyme α amylase and glucoamylase[29].

Measurement of iodine complexing ("Blue values")[7]. The dried starch (50 mg) was dissolved by heating in dimethyl sulphoxide (5 mL), and the volume was adjusted to 100 mL with water. An aliquot (2-5 mL) of the solution was transferred to a 50 mL flask, followed, in succession, by sodium chloride solution (1.0 *M*, 1 mL), water (40 mL), iodine solution (1mL) containing 2.0 mg of iodine and 20 mg of potassium iodide), and additional water to make a total volume of 50 mL (the concentration of starch was~2 mg/dL). After 30 min, the absorbance at λ = 600 nm was measured with the spectrophotometer, using a 10-mm cell; the blue value expressed as 4A/C (mg/mL).

Conclusion

In conclusion, starch from the tubers of *Curcuma aeruginosa* is a highly amylase starch with less crystallinity because amylopectin is responsible for the ordered nature of starch.

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