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## CONFORMATION OF STARCH POLYSACCHARIDES IN SOLUTION. HIGH-FIELD NMR INVESTIGATIONS AND THEORETICAL CALCULATIONS

### Abstract

Interest in the structure and conformation of starch polysaccharides - having either academic or industrial motivation - is not diminishing. Aqueous solutions challenge new experimental techniques.

Results of high-field NMR measurements are reported related to maltooligomers. Unambiguous spectral assignments were obtained and it was found that all monomeric subunits have the  ${}^4C_1$  conformation.  ${}^{13}C$  relaxation times ( $T_1$ ) show remarkable flexibility (0.3 sec at 80°C). Minimization of non-bonded energy of helical segments of various lengths (up to DP 24) resulted in left-handed helical structures. Glycosidic carbon-proton coupling constants ( ${}^3J_{CH}$ ) corroborate with the predicted dihedral angles.

### Introduction

Although the main structural features of starch and related saccharides are known for many years, research interest in this field - having either academic or industrial character - does not diminish. There are several reasons for that:

1. The problem of fine structure is continuing to be the subject of investigation and speculation. The energy-storing biopolymers amylopectin and glycogen are typical examples. Both polysaccharides are highly branched having high complexity as compared to linear chains. But even in case of amylose, having a predominantly linear structure, conformation in solution, double to single helix formation and chirality have been the subject of numerous recent publications.
2. Improvements in experimental techniques have been remarkable, first of all in instrumental analysis and enzymic methods. In structure determination new results revealed by high intensity X-ray techniques, dynamic light-scattering experiments. Solid state  ${}^{13}C$ -NMR sufficient resolution for carbohydrate studies and is now used to investigate aqueous aggregation and gelation of amylose. By sophisticated multidimensional pulse techniques high-field NMR measurements in liquid phase give information on solution conformation of various oligo- and polysaccharides.

3. Theoretical calculations and graphical visualization help in structure investigation and evaluation of complex experiments. High-speed work stations are available and semi-empirical methods for energy calculations are now well parametrized for the carbohydrate field.

In this paper we shall concentrate on approaches offered by high-resolution, high-field NMR interpreted by insight derived from theoretical calculations.

Theoretical calculations were introduced already in the 70-es to unveil the energy constraints that govern three-dimensional molecular structures. These were used at first to supplement diffraction analysis. With the advent of fast computers they have become indispensable tools of present-day analytical methods. Computational methods were developed simultaneously to treat even macromolecules including solvent interactions and it is also possible to model their dynamic behavior.

Investigation of starch related polysaccharides in solution, especially in water, is connected with the increased interest in the biological functions of carbohydrates.

NMR techniques were developed relatively lately and were entered into the starch field only recently. The first, and perhaps most important feature revealed by NMR was the increased mobility of oligo- and polysaccharide subunits. Relaxation time determinations were evaluated in terms of the microdynamics and local correlation.

### Results and discussion

In connection to all these new developments we report now on our studies on the average conformation of amylose in aqueous solution.

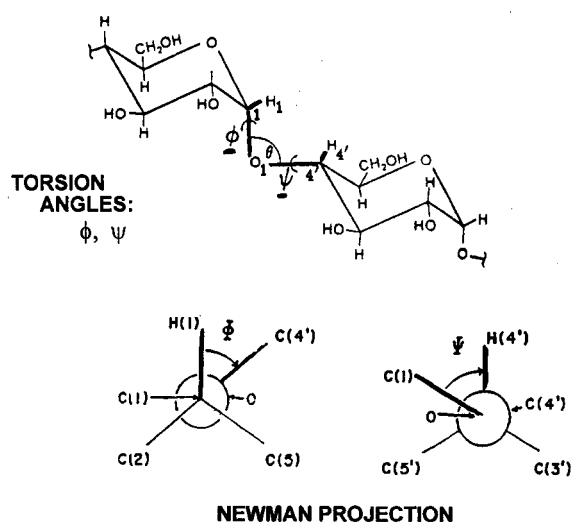


Fig. 1.

High resolution  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectroscopy was chosen as a method because it results in quantities that can be compared to predicted values of theoretical calculations. We concentrated on a conformation dependent NMR parameter: the three-bond glycosidic coupling constants ( $^3J_{\text{CH}}$ ).

Their dependence on the torsion angles is well understood.

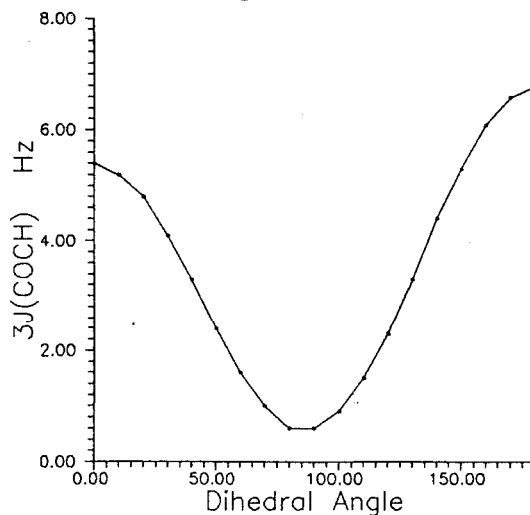


Fig. 2.

Commercial samples of maltooligomers were used for this study and a monodisperse amylose (DP 4800) kindly submitted by prof. Beate Pfannemüller.

Table 1

$^1\text{H}$ -NMR chemical shifts [ppm] and vicinal coupling constants [Hz] of amylose (DP 4800) determined at 600 MHz and 80°C in  $\text{D}_2\text{O}$ . Chemical shifts are referred to TMS.

Atom	$\delta(^1\text{H})$	$M^a$	$^3J_{\text{HH}}$
1	5.350	d	3.9 ( $J_{12}$ )
2	3.621	dd	9.9 ( $J_{23}$ )
3	3.938	dd	8.9 ( $J_{34}$ )
4	3.620	t	9.7 ( $J_{45}$ )
5	3.819	ddd	2.1 ( $J_{56a}$ ) 5.1 ( $J_{56b}$ )
6 <sub>a</sub>	3.863	dd	-12.2 ( $J_{6a6b}$ )
6 <sub>b</sub>	3.791	dd	

<sup>a</sup> d - doublet, t - triplet, dd - podwójny dublet, itd.

The following results were obtained:

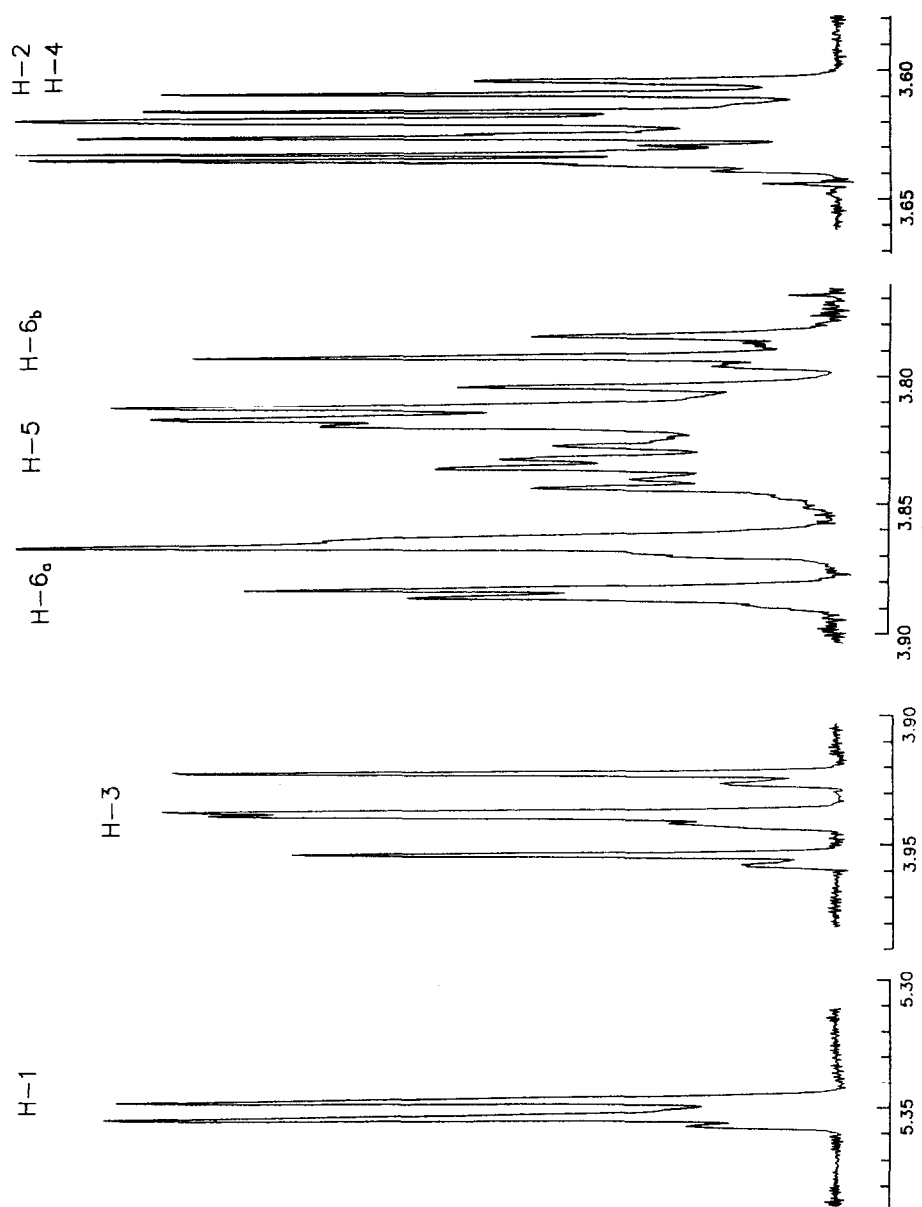


Fig. 3.

Unambiguous assignment of the  $^1\text{H}$ -NMR spectrum of the amylose sample was possible only at 600 MHz. Fig. 3 shows all the multiplets of the one-dimensional spectrum. Precise values of proton chemical shifts and coupling constants were derived by analyzing the multiplets by spin-simulation.

These coupling constants are compatible only with the  $^4\text{C}_1$  conformation of the glucose subunit in each subunit of the oligomer.

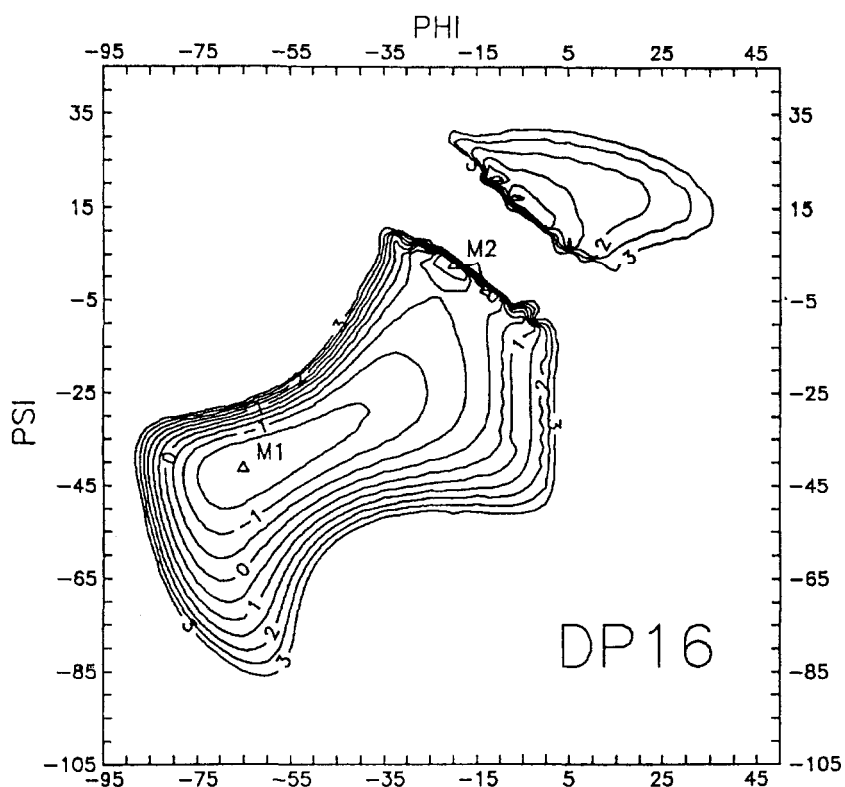


Fig. 4.

Using program CAOS (developed in our Institute) the non-bonded conformational energy of several maltooligomers with various Degree of Polymerization (DP 7, 8, 16, 24) has been calculated in helical conformation as a function of the glycosidic torsion angles (relaxing the hydroxymethyl side chains and without hydrogen bonding or solvent effect). For DP 16 it is represented on Fig. 4 as a relief contour map. M1 and M2 denote the two low-energy minima. Energy maps for different maltooligomers have similar character, only the energy values depend on the DP.

Note that left-handed helices (corresponding to the contour levels at negative  $\Phi$  and  $\Psi$  values) are less energetic than the right-handed ones and are separated from each other by a high potential barrier. The lowest energy right-handed conformation is denoted by M3.

Our experiments verified that:

The NMR parameters are practically independent of the degree of polymerization and they are identical with the chemical shifts of monodisperse amyloses of low (DP 35) and of high molecular weight (DP 4800) - results obtained some years ago in collaboration with dr. Beate Pfannemüller.

Proton-carbon coupling constants of the C-O-C-H type depend on the absolute value of the torsion angle. So they can be used to evaluate the average torsion angles. For  ${}^3J_{CH}$  values found in maltoheptaose (4.6 and 5.3 Hz)

$$4.6 \text{ Hz} \rightarrow \Phi_H(\text{H-1-C-1-O-C-4}') = -26^\circ \text{ (or } 140^\circ) \text{ and}$$

$$5.3 \text{ Hz} \rightarrow \Psi_H(\text{H-4'-C-4'-O-C-1}) = -4^\circ \text{ (or about } 153^\circ)$$

where values in parentheses follow from the double-valued nature of the function (and will be disregarded from energetic considerations). These torsion angles describe a conformation that is not far from the minimum M2.

In case of amylose we found a value of 4.4 Hz for  ${}^3J_{Cl,H-4'}$ . The corresponding  $\Psi_H$  value ( $-26^\circ$ ) indicates a similar conformation, not essentially different from that of DP 7 discussed above.

In general in saturated flexible molecules both chemical shifts and vicinal coupling constants are time-averaged values over all possible conformations in solution. Longitudinal relaxation times ( $T_1$ ) of maltooligomers and amylose prove that local motion of the subunits is very effective for relaxation (despite of the long linear chain) resulting in values of the order 0.2 s (at 75.5 MHz).

Our results determined at 70°C are complementary to those of professor Brant published recently for room temperature and indicate the damping function of the solvent to the local motion.

## Conclusions

On the basis of above findings the following conclusions have been drawn:

The average conformation of all glucose subunits in amyloses and various maltooligosaccharides in pure, neutral D20 solution at 80°C can be characterized as the  ${}^4C_1$  conformation.

NMR parameters (chemical shifts, coupling constants) vary only slightly with DP (for the DP values studied). Local motion of the subunits is efficient enough to result  $T_1$  values of about 0.3 s.

Lowest energy minima of single helices having constant pitch but various lengths (between DP 7 and 24) as calculated by the HSEA method were found to have minima in the left-handed region (both the extended and the compact forms).

Glycosidic  $^3J_{CH}$  coupling constants and nOe values observed in aqueous solutions are compatible with conformational parameters ( $\Phi_H$ ,  $\Psi_H$ ) being not far from the compact type minimum (M2). The importance of M2 over M1 is enhanced with increasing DP.

These data support the representation of amylose conformation in solution as a randomly broken helical chain.

#### KONFORMACJA POLISACHARYDÓW SKROBIOWYCH W ROZTWORZE. BADANIA W WYSOKOPOŁOWYM NMR I OBLICZENIA TEORETYCZNE

##### Streszczenie

Nie maleje zainteresowanie strukturą i konformacją polisacharydów skrobiowych. Ma ono motywacje akademickie i przemysłowe. Roztwory wodne pozwalają na stosowanie nowych technik badawczych.

W pracy podaje się wyniki pomiarów maltooligosacharydów w zakresie magnetycznego rezonansu jądrowego w wysokim polu.

Czasy relaksacji ( $T_1$ ) atomów węgla ( $^{13}C$ ) są wysoce elastyczne (0.3 s. w 80°C).

Minimalizacja energii nie wiążącej dla segmentów heliksu o różnej długości (do DP = 24) sugeruje lewoskrętne struktury. Stałe sprzężenia glikozydowych atomów węgla ( $^3J_{CH}$ ) zgadzają się z przewidzianymi kątami wiązań. ☒