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HIGH RESOLUTION 13C- AND 1H- NMR STUDY OF STARCH GEL

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Nuclear magnetic resonance (NMR) spectroscopy was used to study molecular changes during storing and forming spatial network in concentrated starch solution in D_2O. The concentration and temperature effect in chemical shift of proton and carbon signals in NMR spectra allowed to determine the thermodynamic parameters of helix-coil transition.

INTRODUCTION

The starch solutions obtained on boiling the water suspension of starch in normal conditions, show a tendency to the aggregation on cooling them, and in the appropriately high concentration they have ability to form a fixed spatial structure [19]. This process known as retrogradation of starch, determines many qualitative parameters of food products of starch processing. The results of the first study by Katz show that one of the effects of structural change in starch during retrogradation is a partial recrisstalization of polysaccharide. It was confirmed in many later experimental works, based on X-ray diffraction [5, 13, 14] and calorimetric methods [4, 9, 17].

NMR spectroscopy is known as a universal method to study polymer systems [1-3, 6-8, 11-12, 16]. It was shown [12] that a sensitive parameter of conformational changes in polysaccharide compounds is the chemical shift of the resonance frequency in NMR spectrum. This method should be useful to study wheat starch gel in retrogradation process that involves structural changes in the spatial network of the forming gel. Conformational changes considered as a local molecule convert from a random coil to a helix affect the pattern of electron distribution about the linkage, and therefore can be detected by NMR as changes in the electronic shielding of the atoms involved in the linkage.

MATERIALS AND METHODS

A commercial wheat starch was used throughout with main contents of amylopectin (above 90%). As a solvent the D_2O was used of 99.84% excitation. Homogeneous solutions of starch were prepared by long time boiling of the

polymer suspension and then by cooling it to the appropriate temperature. The measurements were realized for the starch concentration range 0.2-1.2 g/cm³ and for the temperatures: 20-80°C.

NMR experiments were carried out on a pulse JEOL FX-90Q spectrometer. ^{13}C and ^{1}H-NMR spectra of starch gel were taken on at the basic frequency of 22.6 MHz and 89.55 MHz, res. The spectral width of ^{13}C spectra was 5.0 Hz and 1000 Hz for ^{1}H spectra, the repetition time was 0.3 and 7.0s and the accumulation number was 25000 and 10, respectively. The chemical shifts, quoted in ppm from tetramethylsilane (TMS), were measured by reference to external dioxane. Chemical shifts of proton bands were read out after 1 and 24 hours of ageing gel, the shifts of carbon bands were read out after 24 hours of storeing the solution at a given temperature.

RESULTS

The influence of two basic factors was studied: the concentration of starch and the storing temperature. It was not possibile, in respect of ^{13}C-NMR technics, to measure a kinetic of the evolution of spectra during ageing starch gel, therefore we restricted the measurements to analysis of the metastable starch gel structure formed after 24 hours of storeing. The temperature dependence of chemical shifts of the main six resonance signals ascribed to carbon atoms C1-C6 in ^{13}C-NMR spectra of wheat starch gel illustrates Table.

T a b l e . Chemical shift variation of carbons in ^{13}C-NMR spectra of wheat starch gel of concentration 0.1 g/cm³

Temp. range, °C	$\Delta\delta/\Delta T$ 10^3, ppm/°C					
	C-1	C-2	C-3	C-4	C-5	C-6
20-40	32.0	32.0	32.0	36.0	32.0	35.0
40-60	5.0	8.5	5.0	19.0	9.5	9.0
60-80	1.0	3.5	1.0	10.0	4.0	6.5

When the temperature increased, the signals of all carbon atoms in NMR spectra exhibited downfield chemical shifts. The effect was non linear. A rapid change was observed in the slope value $\Delta\delta/\Delta T$ above 40°C, that may be a consequence of rapid conformational change of polymer chains. A parameter indicating the formation of helix conformation of polysaccharide chains is the ratio of shifts $\delta C1/\delta C4$ [12]. In this case the parameter increased from 0.1 in the range of higher temperatures 60-80°C to the value of 0.89 below 40°C, which proves a significant increase of the spiral forms contribution in the lower temperatures, 20-40°C.

The concentration effect has been observed for starch gel in ^{1}H-NMR spectra (Fig. 1).
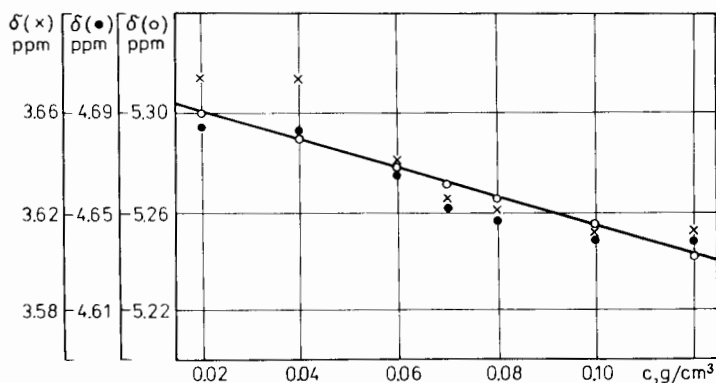

Fig 1. Concentration effect on chemical shift in ^{1}H-NMR spectra of wheat starch gel: (●) — anomeric proton band, (○) — hydroxyl protons band, (x) — other protons band

In the first approximation the concentration dependence of all three observed signals, due to anomeric proton, hydroxyl group and the other nonhydroxyl protons of starch molecule, may be assumed as linear. This linear approximation is the best for anomeric proton signal.

Taking into account that in the formation of the spatial gel network the elementary joints of polymer chains are bihelical associates that are stabilized by hydrogen bonds, then the conformational changes involving the formation of new hydrogen bonds may be treated as small perturbation in the screening of nuclei, observed as the changes in chemical shifts in ^{1}H-NMR spectrum. In this approach the obtained value of signal chemical shift may be expressed as the sum:

$$\delta = \frac{1}{n} \Sigma\, n_i\delta_i \qquad (1)$$

where: n_i-the number of nuclei in the i-th conformational state, with the shift δ_i, n-the total number of all proton nuclei in the system.

Expanding this formula into the term related to monomer units, not involved in site formation, and the terms describing the contribution of the nuclei built in the network joints with different functionality f and different length of units, to the chemical shift, we come to relation:

$$\delta = \frac{1}{n} (n_o\delta_o + \sum_i^w \sum_k^{f_i} 0.5\, n_{ik}\delta_{ik}) \qquad (2)$$

n^{ik}-the number of nuclei of the k-th segment in the i-th joint w.f — the number and functionality of the joints in the system.

According to the above equation, the observed relation between the chemical shifts of proton signals and starch concentration may arise from the formation of new spatial lattice sites of the content and functionality determined by the polymer concentration. Assuming for protons built in the joints the same value of δ_w, independent of the site functionality and unit lengths we can rewrite equation 2 as;

$$\delta = \frac{n_o}{n} \delta_o + \frac{fw}{2} \frac{n_w}{n} \delta_w, \tag{3}$$

where: $fw/2$ — the number of lattice segments in gel unit volume.

Let's denote the number of protons of a given segment built in the network sites by n_w, the number of protons not involved in site formation by n_{os} and the number of all protons of a given group in a segment by n_s. The following relations hold: $n_s = n_{os} + n_w$, $n_o = fwn_{os}/2$, $n = fwn_s/2$. Assuming this notation we come to the following expression for the chemical shift of the band of a given proton group as a function of relative number of protons built in the joints:

$$\delta = \delta_o - \frac{n_w}{n_s} (\delta_o - \delta_w). \tag{4}$$

Taking for the extreme concentration values; for $c \rightarrow 0$, $n_w/n_s = 0$, and for $c = 1.55$ g/cm^3 (the starch concentration in crystalline phase) $n_w/n_s = 1$, we can determine the signal positions, characteristic for amorphous and crystaline phase of the starch, respectively, as well as the relative number of protons involved in the joints of gel network. For example the relative number of anomeric protons built in the network joints, obtained for the gel of $c = 0.12$ g/cm^3 is $n_w/n_s = 0.078$.

The temperature effect was observed in ^{1}H-NMR spectra of wheat starch gel (Fig. 2). Making allowancs for temperature change of standard signal, there the positions of all three proton signals in relation to each other were analysed. As a reference, a midpoint of nonhydroxyl protons band was choosen.

The increase in OH group protons screening observed in high temperatures may suggest developing dissociation of hydrogen bonds. The sigmoidal shape of the temperature dependence of hydroxyl proton screening proves the presence of two main conformational states which differ in the degree of hydrogen bond dissociation. Thus the helix-coil transition may occur involving chemical shift change of OH group. Quantitative analysis of the transition may be carried out assuming the existence of two positions of band; characteristic for the maximum hirality in low temperatures, δ_n and for coil state in high temperatures, δ_c. Then, the chemical shift at a given temperature is described by:

$$\delta = \delta_n - (\delta_n - \delta_c) \frac{n_c}{n}, \tag{5}$$

where the relative contribution of monomer chain units in the coil state is determined by:
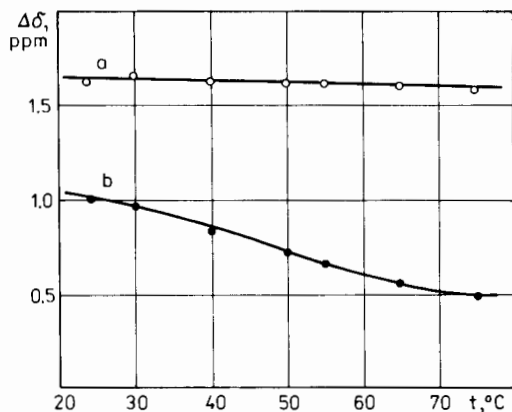
$$\frac{n_c}{n} = (1 + s^m)^{-1}. \tag{6}$$

Fig 2. Temperature effect of relative chemical shift of anomeric proton band (○) and hydroxyl proton band (●) refered to the midpoint of other protons band in ^{1}H-NMR spectra of wheat starch gel of concentration 0.1 g/cm³

The equilibrium constant s^m for monomolecular reaction of cooperative group transition of m subsequent units into the region of helix conformation is [20];

$$s^m = \exp[-(\Delta H - T\Delta S)/RT]. \tag{7}$$

By fitting this equation to experimental data, we obtained the changes in the enthalpy of transition $\Delta H \simeq 19.2$ kcal/mol and for the entropy, $\Delta S \simeq 59.7$ kcal/molK; the enthalpy of transition per one mol of monomeric units, assuming the most probable helix form for starch chains, consisting of six residue per pitch, is 3.2 kcal/mol. This value is closely adequate to the value of enthalpy, predicted for six residue monohelikal conformation [15]. The temperature of this transition in starch gel was $T_c = 321.5$ K $= 48.5°C$.

The kinetic constant of the helix — coil transition may be estimated from the Gutowsky, Holm equation:

$$k = 2^{\sqrt{2}}(f_n - f_c)/2, \tag{8}$$

where: f_{n-fc} is the diffrence in nuclear resonance frequencies in Hz, determined for the helix form and coil state;

$$f_n - f_c = (\delta_n - \delta_c)\, 10^{-6}\, f_o, \tag{9}$$

where: $f_o = 90$ Hz

The kinetic constant found in this way is of about 35 s^{-1} that points to a high rate of conformational transitions in starch chains. It may explain why no

changes in chemical shifts were observed in ^{13}C and ^{1}H-NMR spectra during the process of gel ageing.

CONCLUSIONS

Water solutions of wheat starch, homogenized in the boiling temperature, are insert by cooling them, into the region of worse solvent quality, specified by parameter characterizing the polymer-solvent interaction [10];

$$h = 0.5 - \text{ø}(1 - \text{®}/T), \qquad (10)$$

® — Flory's temperature, defined by the ratio of the enthalpy ΔH_m of the solution to its entropy ΔS_m, is the temperature in which the chemical potential of polymer-solvent interaction is equal zero. Thus the mixing heat ΔS_m is compensated by the increase of conformational entropy ΔS_m. In temperature below R the energetic agent is not compensated by entropy agent, therefore the interaction between polymer segments dominates over the polymer-solvent interaction. In these terms it may occur as well intramolecular association (helix forming) as intermolecular association, involving cristallization or (and) network forming in the system. The first effect is observed by chemical shift changes in carbon and proton magnetic resonance. Analysis of the relative change of signal positions of C-1 and C-4 in ^{13}C-NMR spectra showed an evident increase of the degree of chain spirality in wheat starch below 40°C.

The temperature change of the proton hydroxyl screening, in ^{1}H-NMR spectra of starch solution in D_2O, proved the conformational transition in the temperature $\simeq$ 50°C. In lower temperatures the intramolecular polymer-polymer interactions realised by hydroxyl bonds between the nearest units in the helix, are predominant over interaction of solvent molecular with OH(2) and OH(3) group in starch monomer unit, which are attributed to stabilization of hiral structure in the chain [9]. Respecting to this type of interactions, the temperature of phase transition $T \simeq 322K$ may be identified with R — temperature for water solution of starch. The kinetic of that postulate transition is not detected by measurements of ^{13}C and ^{1}H-NMR spectra of starch due to very small time constant.

Acknowledgement

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BADANIA KLEIKÓW SKROBIOWYCH METODĄ WYSOKOROZDZIELCZEJ SPEKTRO-SKOPII ¹H- i ¹³C-NMR

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Streszczenie

Kleiki skrobiowe sporządzone podczas przechowywania roztworów homogenicznych otrzymanych ze skrobi pszennej zagotowanej w D_2O badano przy użyciu pulsowego spektrometru JEOL FT-90Q NMR. Widma ¹³C-NMR zarejestrowano po 24 h przechowywania kleików. Wzrost temperatury przechowywania kleików powoduje niskopolowe przesunięcie sygnałów pochodzenia węglowego w widmie NMR. Stosunek przesunięć C1/C4 związany z tworzeniem helisy w łańcuchu polisacharydu wskazuje na przejście konformacyjne w zakresie 40-60°C. Bardziej szczegółowa analiza zależności temperaturowej sygnałów od protonów hydroksylowych obserwowanych w ¹H-NMR dowodzi przejścia konformacyjnego w temp. ok. 50°C. Zmiany entalpii i entropii przejścia wynoszą 19,2 kcal/mol i 59,7 kcal/mol K res.

Dla wszystkich badanych kleików skrobiowych przechowywanych w stałej temp. 20°C zaobserwowano zależność pomiędzy stężeniem a przesunięciem chemicznym dla wszystkich sygnałów ^{1}H-NMR. Efekt ten przypisano liniowej zależności pomiędzy stężeniem polimeru i stopniem fazy krystalicznej istniejącej jako podwójna helisa w kleiku skrobiowym.