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Molecular mobility of polysaccharide chains in starch investigated by two-dimensional solid-state NMR spectroscopy

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Slow molecular motion of polysaccharide chains in native starch and in retrograding starch gels was investigated by one- and two-dimensional exchange solid-state NMR spectroscopy. In native starch, no evidence of molecular motion of the polysaccharide chains on a time-scale of seconds was found. Molecular motion of polysaccharide chains during retrogradation on a time-scale of milliseconds was clearly visible from line broadening in the one-dimensional spectra. The spectra indicated motions with correlation time (τ_c) of about 100 μs. Ultraslow motions with τ_c of tens of milliseconds were detected with stimulated echo and two-dimensional exchange NMR. Results showed that the retrogradation process occurs with a very wide distribution of correlation times. This NMR approach can be used in studies examining molecular mobilities in a wide range of complex and polymeric materials in relation to their physico-chemical properties and behaviour. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Native starch is present in the form of semi-crystalline granules composed of two types of glucose polymers: essentially linear amylose ((1→4)-α-D-glucan) and highly branched amylopectin ((1→4)-α-D-glucan with α-(1→6) branch points) (French, 1984). The average crystallinity of starch granules is about 30% (Zobel, 1992). The structure of native starch was primarily probed by X-ray diffraction, which has led to a detailed description of polymer chain packing in the crystalline part (Imberty *et al.*, 1988; Imberty & Perez, 1988).

An interesting phenomenon in starch is gelatinization. It is an order–disorder transition occurring when starch is heated in water to temperatures in excess of 50–60°C. It is accepted that during this process the native granular structure is disrupted (Zobel, 1992). At high starch concentration an opaque visco-elastic gel is formed. On ageing the gel returns to the semi-crystalline state in a process called retrogradation. Since starch is used as a structuring ingredient in many foods, knowledge of the physico-chemical background of the functional behaviour of starch and starch polysaccharides is important. Numerous studies have been reported on gelation and retrogradation using differential scanning calorimetry (DSC) (Donovan, 1979; Russel, 1987a, b), X-ray diffraction (Liu *et al.*, 1991; Jenkins *et al.*, 1994), FT-IR (Goodfellow & Wilson, 1990) and NMR (Morgan *et al.*, 1992; Wu & Eads, 1993). These reports focused mainly on the growth of a solid-like component in gels. Likewise, the molecular mobility of highly mobile fractions in starch-based products has been investigated (Callaghan *et al.*, 1982; Wu & Eads, 1993). However, evidence of ultraslow motions on a time-scale of milliseconds/seconds that could govern the retrogradation process could not be found.

Among the many experimental techniques employed in studying starch structure, solid-state NMR has proved to be particularly successful (Gidley, 1992). The combination of the high resolution in the spectra obtained by magic-angle spinning (MAS) (Andrew *et al.*, 1958; Schaefer & Stejskal, 1976) and signal enhancement with cross-polarization (CP) (Pines *et al.*, 1973) yields line shapes that reveal information at the sub-molecular level. Moreover spectral deconvolution allows estimation of the double helix content in native

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starches (Gidley, 1992). Molecular mobility is another aspect of the organization of polymer chains in native starch and this has been studied by ^1H T_1 and $T_{1\rho}$ (H) relaxation experiments (Froneman & Reynhardt, 1991). Froneman & Reynhardt concluded that the α-(1→6) branching of the amylopectin chains results in higher relaxation rates, implying fast molecular reorientations in the branches. Apparently molecular motions are due to uniaxial reorientations of CH_2OH groups. The rate of these motions at 27°C was found to be about 21 kHz. Recently we reported a study on molecular mobility in native starch (Kulik *et al.*, 1994a) in which information about the molecular mobility of the polysaccharide chains was obtained from the width of the NMR proton wideline recorded in a two-dimensional heteronuclear ^1H–^{13}C (2D) wideline separation (WSE) experiment (Schmidt-Rohr *et al.*, 1992). The width of this line (25 kHz) was the same for every distinct carbon site. Such a value is typical of rigid solids and indicates the absence of molecular motions within the polysaccharide chains on a time-scale faster than microseconds. The question remains whether in native starch molecular motion of polysaccharide chains takes place at all. Motion on the time-scale slower than microseconds can be probed by 2D exchange NMR (Ernst *et al.*, 1987). This technique has been applied successfully in studies of molecular reorientation in synthetic polymers with correlation times in the range $0.1\,\text{ms} < \tau < 10\,\text{s}$ (Schmidt-Rohr & Spiess, 1994). We now demonstrate the applicability of this technique to the study of the mobility of polysaccharide chains in starch materials.

EXPERIMENTAL PROCEDURES

NMR background

Since static ^{13}C solid-state NMR experiments are non-standard, the relevant features of this technique will be briefly summarized. Detailed description can be found elsewhere (Schmidt-Rohr & Spiess, 1994). ^{13}C NMR spectra are governed by structure-dependent magnetic shielding of the nuclei, known as the chemical shift (Abragam, 1961). In solids this interaction is not averaged by fast isotropic molecular motions as it is in the case of liquids. Consequently, static ^{13}C spectra exhibit wide, overlapping lines for carbons in different chemical environments. In order to obtain higher spectral resolution in ^{13}C solid-state NMR, this anisotropic interaction is averaged by MAS (Schaefer & Stejskal, 1976). However, the increase in spectral resolution achieved by MAS is at the expense of information about molecular motion, which is absent in the spectra. Static spectra do yield this information since the NMR frequency depends on the orientation of a molecular unit relative to the external magnetic field B_0 given by (Abragam, 1961):

$$\omega_0 = \omega_L + \frac{\delta}{2}(3\cos^2\theta - 1 - \eta\sin^2\theta\cos 2\Phi)$$

where ω_L is the Larmor frequency including the isotropic chemical shift and δ is the strength of the anisotropic coupling. The asymmetry parameter η characterizes the deviation of the chemical shift tensor (CST) from axial symmetry and the polar angles θ and Φ specify the orientation of the magnetic field vector B_0 in the principal axes system of the tensor. The angular dependence of the NMR frequency, shown in equation (1), is used in monitoring molecular dynamics, via NMR spectra (Schmidt-Rohr & Spiess, 1994) and relaxation rates (Abragam, 1961).

Information about the time-scale of ultraslow motions is available from the 2D exchange experiment (Ernst *et al.*, 1987). In this experiment, molecular orientation is measured via the NMR frequency (equation (1)) before and after a mixing time t_m during which reorientation can take place. The resulting 2D spectrum, $S(\omega_1, \omega_2, t_m)$, represents a two-time distribution function (Wefing & Spiess, 1988). This function describes the joint probability density of finding a molecule with frequency ω_1 corresponding to the initial orientation before t_m and frequency ω_2 corresponding to the final orientation after t_m. In the absence of motion, the frequencies ω_1 and ω_2 are the same and the spectral intensity is confined to the diagonal $\omega_1 = \omega_2$. In the case of large-angle reorientation about the same well-defined angle, the 2D spectrum displays characteristic exchange ridges in the form of higher-order curves (Schmidt-Rohr & Spiess, 1994). Random motions or reorientations about ill-defined angles result in a 2D spectrum without sharp contours. Thus, 2D exchange spectra can discriminate between different motional mechanisms.

NMR experiments

The NMR experiments were carried out on a Bruker MSL-300 spectrometer operating at a ^{13}C resonance frequency of 75.47 MHz. All spectra were recorded with CP (Pines *et al.*, 1973), applying a contact time of 2 ms in a double-resonance probe at 20°C. Stimulated echoes were recorded with a $90°$–t_1–$90°$–t_m–$90°$ acquisition pulse sequence, which was also used in the 2D exchange experiment, where t_1 and t_m are the evolution time and the mixing time, respectively. The spectra were obtained by Fourier transformation starting at the echo maximum (Hentschel *et al.*, 1984). The 2D spectra were taken with 30 to 64 t_1 increments of 32 μs. Typically about 500 scans were averaged. The ^1H 90° pulse was 4.5 μs. Fourier transformation of the data and data analysis was performed on a Silicon Graphics computer system using a modified PV-Wave software package.

Samples

A-type waxy maize starch (Amioca 85) was obtained from National Starch (Zutphen, The Netherlands) and was used without any chemical treatment. The moisture level in the sample during NMR measurements was approximately 12%, as determined by vacuum oven drying. Starch was gelatinized in an NMR rotor in a 60% mixture in water. The rotor was heated in boiling water for 15 min until the sample became translucent. After gelatinization the sample was cooled in a water bath at ambient temperature for 5 min and measured. Drying during retrogradation was prevented by a thin layer of silicone wax between the sample and a tight-fitting cap.

RESULTS AND DISCUSSION

NMR on native starch

Figure 1 shows the ^{13}C CP spectra of starch with and without MAS. The advantage of spectral resolution with MAS is clearly visible and peaks corresponding to different positions in the glucose units can be distinguished (Gidley, 1992). However, as mentioned above, spectra exhibiting isotropic chemical shifts do not yield information on molecular motions. Line shapes obtained under static conditions are broad and structureless. The signals corresponding to carbons in various chemical environments (C-1–C-6 of the glucose residues) overlap and cannot be resolved. Broad lines indicate the absence of large-amplitude motions with rates exceeding the width of the spectrum, i.e. above 8 kHz. This is consistent with previous 2D WISE measurements indicating the absence of molecular mobility at rates above 25 kHz (Kulik *et al.*, 1994a).

In order to check whether molecular motion occurs on a longer time-scale, 2D exchange NMR techniques were applied. Figure 2 shows a stack plot of the 2D exchange spectrum obtained for $t_m = 1$ ms. The intensity is confined to the diagonal, suggesting the absence of molecular mobility. Much better representation of data is provided by the contour plot shown in Fig. 3. Since the intensity is restricted to the diagonal it can be concluded that no molecular reorientations take place on a time-scale of 1 ms (mixing time). The existence of even slower molecular motions can be checked by applying longer mixing times. Figure 3 shows the contour plot of the 2D experiment with a mixing time of 2 s. The contour plot is almost identical to the spectrum recorded for 1 ms. The slight broadening of the diagonal is due to the spin diffusion process (Edzes & Bernards, 1984).

Our experiments indicate the absence of molecular mobility on a time-scale of seconds in native starch. It is well known from X-ray studies (Zobel, 1992; Jenkins *et al.*, 1994) that side chains of amylopectin are packed in double helices oriented in spherical crystalline zones. The branch points of amylopectin contribute to the amorphous areas located between the semi-crystalline amylopectin zones. These amorphous regions also contain the amylose chains. The backbone mobility in amorphous and semi-crystalline polymers below the melting point depends on the glass transition temperature T_g (McCrum *et al.*, 1967). At this temperature, there is an onset in molecular mobility.

Below T_g large-amplitude backbone motions can be excluded, since the chains are essentially fixed in the structure (Jäckle, 1986). Moreover, molecular motions in the crystalline parts of semi-crystalline polymers occur only above T_g (McCrum *et al.*, 1967). Since at room temperature native starch at 12% moisture is well below T_g, large-amplitude motions can be excluded (Zeleznak & Hoseney, 1987) both in the amorphous and in the semi-crystalline part. Our results corroborate this picture and show that theories regarding synthetic polymers are also valid for biopolymers. Apparently, due to the dense packing of

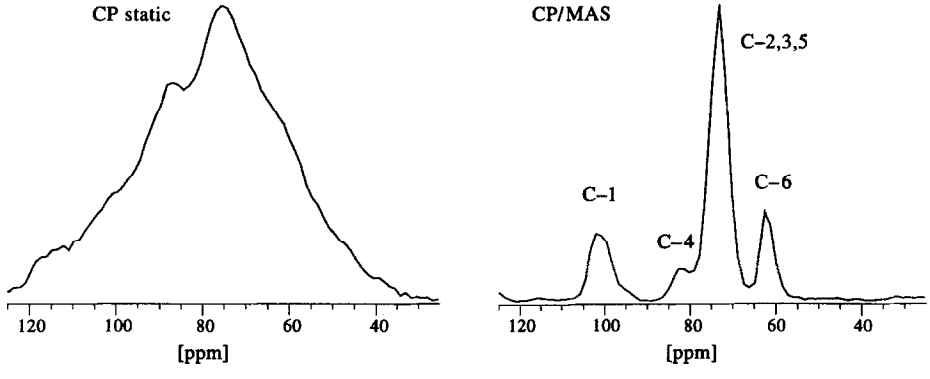

Fig. 1. ^{13}C CP spectra of starch. Left: spectrum obtained under static conditions; anisotropy resulting in broad lines. Right: spectrum under MAS conditions; anisotropic interactions being averaged by mechanical rotation.

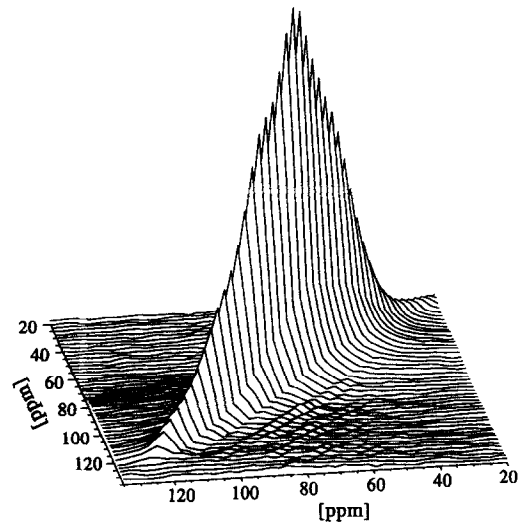

Fig. 2. Stack plot of 2D exchange spectrum of native starch at a moisture level of 12%, taken with t_m of 1 ms.

chains in native starch, molecular motions are intrinsically absent.

NMR on retrograded starch

Figure 4 shows the ^{13}C CP spectra of retrogradated starch recorded as a function of time after gelatinization. (No mechanical rotation was applied.) The line width in the spectrum obtained 30 min after gelatinization is only slightly higher than in the MAS spectrum (cf. Fig. 1). Pronounced line shape changes are observed with respect to the static CP spectrum of native starch (Fig. 1). This is indicative of large-amplitude motions with rates exceeding the width of the CP spectrum, i.e. above 8 kHz. Spectral lines broaden with time, however, even six days after gelatinization significant averaging of the line shape is still visible. It is noteworthy that CP spectra are sensitive to molecular motions that do not average dipolar coupling, i.e. at rates slower than 100 kHz. Thus CP ^{13}C spectra selectively filter the high mobility of polymer chains. Those motions can be detected with the NMR techniques used for liquids (Callaghan *et al.*, 1982; Wu & Eads, 1993). In principle, it is possible to simulate measured 1D spectra and to extract geometric and dynamical information from these spectra. However, this requires knowledge of CST orientation and its principal values. To the best of our knowledge, such data are not available.

It is accepted that during retrogradation first transition from a disordered state (random coil) to an ordered conformation (double helix) occurs. This process is followed by the formation of a physically cross-linked network at the superhelical level (Viebeke *et al.*, 1994). Apparently, these processes occur on an ultraslow time-scale. Similar ultraslow motions

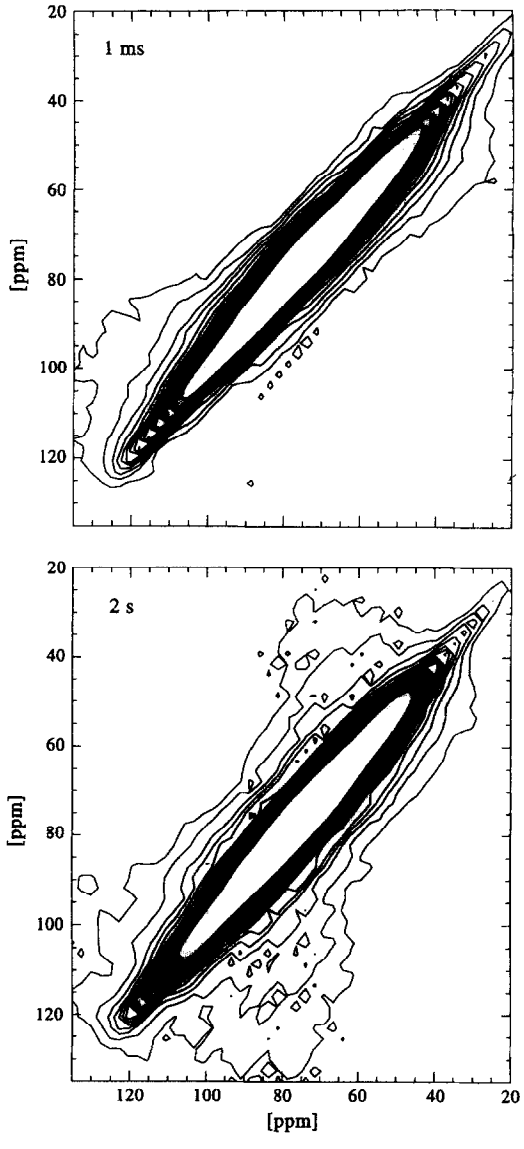

Fig. 3. Contour plots of 2D exchange spectrum taken with the mixing time of 1 ms (top) and 2 s (bottom). The contour lines are linear between 2.5 and 20% of the maximum.

resulting in incomplete motional narrowing have been observed before for the chain motion in the non-crystalline regions of semi-crystalline and amorphous polymers, e.g. poly(ethylene) (Spiess, 1983; Kulik & Prins, 1994), polyamides (Miura *et al.*, 1990) or polymethacrylates (Kulik *et al.*, 1994b). For these polymers, a motional model was derived which postulates the existence of long-lived topological constraints (Spiess, 1983) for the chain motion resulting from the presence of crystallites or hydrogen bonded sheets. Analogously we propose that the molecular motion in starch has a much longer time-scale, as can be deduced from the CP spectra. In order to prove this conjecture experimentally the stimulated echo sequence was used, which can detect ultraslow changes in the anisotropy of the CST resulting from a

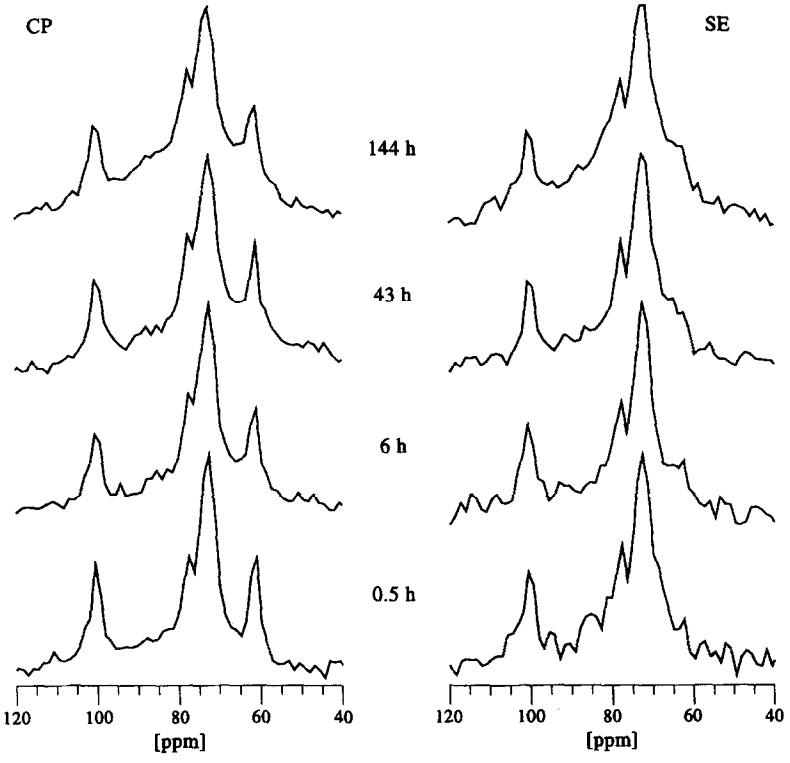

Fig. 4. Static ^{13}C spectra of starch taken after retrogradation as a function of time. Left: CP spectra. Right: stimulated echo spectra obtained for evolution time $t_1 = 100\,\mu s$ with mixing time $t_m = 50\,ms$.

finite lifetime of the motional constraints (Spiess, 1983). The right part of Fig. 4 shows stimulated echo spectra recorded for $t_1 = 100\,\mu s$ and $t_m = 50\,ms$. Pronounced changes are visible for the C-6 signal (CH_2OH group), the intensity of which almost disappears as compared with that of the corresponding CP spectra. This indicates motional heterogeneity with the CH_2OH side groups being more mobile than the polysaccharide backbone. Likewise, the loss of overall intensity is considerable. The ability to detect the stimulated echo directly proves the existence of long-lived constraints on the time-scale of a millisecond.

Changes in the stimulated echo spectra can be shown more clearly in a 2D exchange experiment in which the signal is recorded as a function of the variable time t_1 and not only for a single t_1 value, as in the stimulated echo experiment. Figure 5 shows the 2D spectrum for $t_m = 50\,ms$. The shape of the signal on the diagonal resembles the shape of the CP spectrum. The exchange pattern (off-diagonal intensity) indicates the existence of molecular motions on a time-scale of 50 ms (compare Figs 3 and 5). From the spectra it cannot be concluded whether molecular motion takes place through well-defined jumps. This is because of the overlapping CSTs. Most of the exchange intensity is near the position of the C-6 carbon, corroborating results of stimulated echo spectra. Note that the stimulated echo experiment detects signals from molecular moieties for which $\omega_1 = \omega_2$ (the diagonal in the 2D exchange experiment). Molecular moieties which reorient during t_m contribute to the off-diagonal intensity in the 2D exchange spectrum and are not visible in the stimulated echo spectrum. More detailed

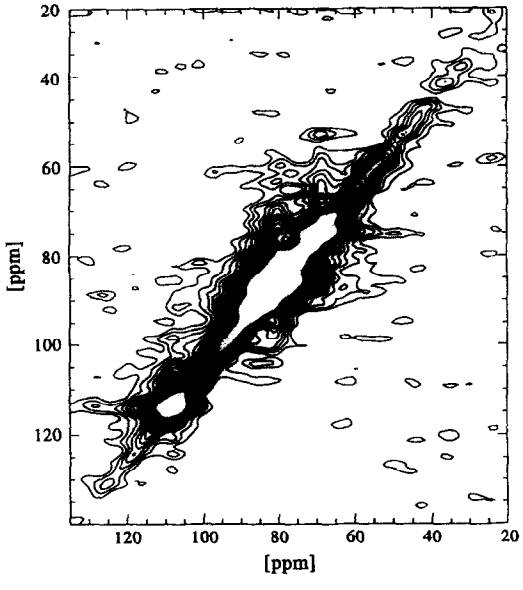

Fig. 5. Contour plot of 2D exchange spectrum of retrogradated starch taken 1–12 h after retrogradation with t_m of 50 ms (lines between 4.5 and 20% of the maximum).

information about geometry of molecular motion cannot be obtained due to the lack of knowledge of CSTs in starch.

Our preliminary data show that it is possible to measure ultraslow motions of polysaccharide chains, which may underlie retrogradation kinetics in starch. These main-chain motions are probably one of the determinants of the macroscopic physico-chemical properties of starch gels. Detailed knowledge of the dynamics and geometry of molecular motion in starch followed by correlation of these motions with functional properties of gels will be a tremendous step towards optimization of applications of starch materials. Work along these lines is in progress.

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