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CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRA OF GROSS PLANT TISSUES CONTAINING STARCH

MASATSUNE KAINOSHO* AND KATSUMI AJISAKA

Central Research Laboratories, Ajinomoto Co. Inc.,

1-1 Suzuki-cho. Kawasaki-shi, 210 Japan

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In recent years, carbon-13 nmr spectroscopy has become a vital technique to obtain dynamic as well as static structural information on the biological molecules.[1] We showed in the previous papers that various compounds including glycosides, sugars, essential oils, and triglycerides (oil) in cells could be directly observed by C-13 nmr spectroscopy of intact plant tissues.[2,3] Most of the compounds so far studied by this technique, however, have been limited to rather small ones which are thought to dissolve in cytoplasms or to localize as oil drops in cells. We have recently found that the intact bulbs of iris and tulip, or a tuber of dahlia gave markedly narrow C-13 signals due to fractans.[4] This is again due to the fact that these polysaccharides are dissolved in the intact plant tissues. Most of the other polysaccharides in plants, on the other hand, exist in more rigid states such as spherulites or fibers. Under these conditions the polysaccharides are expected to move much slower than those in solutions and would therefore give resonances with much broader line widths. In efforts to extend the application of C-13 nmr spectroscopy further in phytochemistry, we have tried to observe high resolution C-13 nmr spectra of various polysaccharides in gross plant tissues. In this communication brief accounts for our studies on various plants containing starch are given.

Intact white potato, which was cut into a cylindrical piece in order to put into an 18 mm nmr sample tube, gave virtually no detectable peaks (*vide infra*). The abundant storage carbohydrate starch was not visible at all under the high

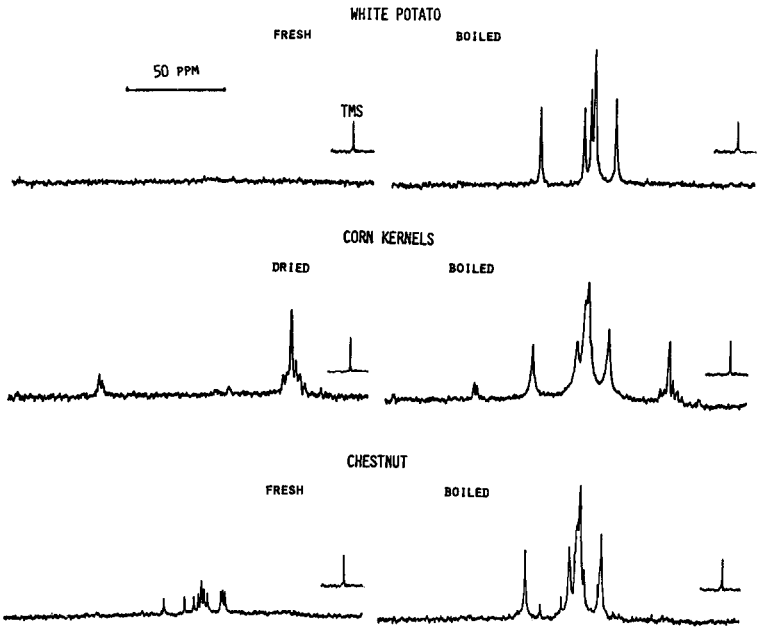

Figure 1. 25.2 MHz Proton decoupled C-13 nmr spectra of white potato (top), corn kernels (middle), and chestnut (bottom). Each of the spectra was run on a Varial XL-100-FT spectrometer equipped with a Nicolet NT-440 multinuclear observing system which allowed us to use 18 mm sample tubes. 5,000 transients after 90 degree pulses were collected for each spectra, with AT = 0.5 sec and SW = 5,000 Hz.

resolution nmr condition. This is obviously because the starch in the intact white potato is in a stiff crystalline state and therefore is immobile. As is well known the crystalline starches are turned to gels on being heated in the presence of excess water, we have examined the effect of heating on the nmr line widths of starches in plants. We therefore measured various starch-rich tissues before and after heated in water. Some illustrative results are given in Figure 1. As the spectrometer conditions were fixed same for each of the tissues, the dramatic effect of heating may thus be evident. All of the boiled tissues, in which starch gelated, gave sharp resonances due to the glucose units of starches, while the tissues before heated showed only small molecules such as oil (corn kernels) or sucrose (chestnut) in cells. In these cases it may be obvious that one could quantitatively analyze the relative amount of starch, oil, or sucrose, without separation procedures.

Let us briefly mention here the state of starch in the intact plant tissues. The electronmicroscopic examination of the starch in white potato has indicated that it localize as the starch granules in cells.[5] Although the molecular structural details of the granules remains yet unclear, it has been known that they compose of tightly packed linear and branched glucans, i.e., amylose and amylopectin, respectively. As the C-13 nmr line widths depend greatly on the motional state of the C-H fragments and they might therefore serve an excellent criterion for the packing states of starches in plants. We have compared the C-13 nmr line widths of starch in an intact white potato to those of the isolated ones. The line widths of starch in the intact potato was found to be too broad to observe by a conventional high resolution nmr equipment, and sharp resonances due to sugars and oil were seen instead (Figure 2A). Note that these signals were not detected after a short accumulation period (see Figure 1A), because sugars and oil exist only a little in white potato, most probably less than 1 %. The C-13 nmr line widths of the freshly prepared starch by a mild procedure were however much narrower (Figure 2B). The same starch but that it was dried overnight at room temperature gave even sharper lines (Figure 2C) when it was dispersed again in water. These observation clearly indicate that the packing state of starch in the *precisely intact* granule is the tightest of all, and the granules isolated even by a mild method, which is often believed to give *intact* starch granules, are looser in their packing states and swell up in water more easily than those in the intact potato. The packing state of the freshly prepared starch granules then becomes looser on

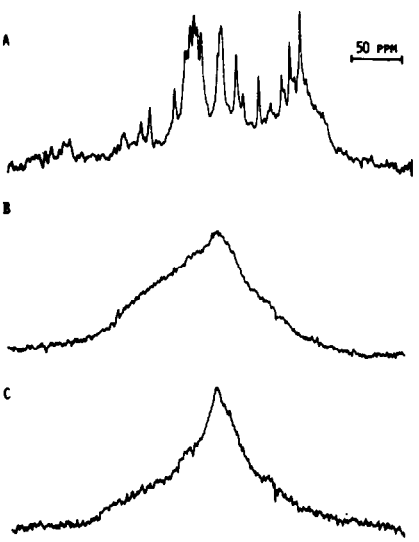

Figure 2. C-13 Nmr spectra of (A) intact white potato; (B) the freshly prepared starch dispersed in water; (C) the same starch except that it was dried overnight in a disiccator and dispersed again in water. All spectra represent the average for overnight runs.

being dried, indicating that the fragile native starch structure is maintained only in the presence of water someway. Nikuni[6] speculated the starch granule in potato might be composed of an extraordinarily huge molecule of starch which tends to break down to smaller ones in the isolation procedures. We believe the C-13 nmr observations described above support his idea in some respect, at least the extreme fragility of starch granules which one might expect from his model.

Recently high resolution C-13 nmr spectra of starches and/or proteins were obtained for various dried plant tissues utilizing a strong proton decoupling to eliminate the dipolar line widths of carbon resonances.[7] In view of rather sophisticated instrumental modifications needed for such experiments, our method in this communication seems to be a good alternative to study the state of starch in various samples. We were able to observe starches contained in various foodstuffs in which the state of starch is considered to be an important issues. By looking at the intensity of starch resonances, the degree of gelation *or* retrogelation of starch in these foodstuffs may readily be seen.[8]

The other insoluble but gelatinizable polysaccharides in plants may in principle be investigated by *in situ* C-13 nmr. For instance, the C-13 nmr spectrum of fresh orange peel (the white part) showed sharp peaks assignable to small sugars, but only faint broad peaks due to pectin appeared. After boiling in water, however, very strong sharp resonances due to pectin appeared, which lost their intensity on standing at room temperature. The latter effect is clearly due to the co-operative association of polysaccharide chains on cooling.[9]

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