Stearic Acid-Starch Interactions as Measured by Electron Spin Resonance[1]

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ABSTRACT

The binding of stearic acid to molecules of starch was explored by using spin probes and electron spin resonance spectroscopy at room temperature, elevated temperatures, and after heating and cooling with wheat, corn, high-amylose, and waxy corn starches. The effect of the location of the spin probe along the fatty acid chain was determined using two spin probes, 16-DOXYL and 5-DOXYL-stearic acid. Methyl 5-DOXYL-stearate was used to determine the effect of replacing the carboxyl group on stearic acid with a methyl ester. Crystallinity patterns for each starch type were verified by wide-angle X-ray diffraction. Immobilization of stearic acid spin probes occurred for all starch types at room temperature before and after heating and cooling. The extent of stearic acid spin probe immobilization was not different in amylose-containing starches at room temperature or heated and cooled samples. However, the immobilization of stearic acid spin probes was less in waxy corn starch than in the amylose-containing starches at room temperature before heating, and the immobilization became even less in waxy corn starch after heating and cooling. Electron spin resonance spectra at elevated temperatures showed stearic acid spin probes were released during heating, but starch-stearic acid interactions again occurred during cooling to room temperature. Spectra for systems of 5-DOXYL-stearic acid and starch or methyl 5-DOXYL-stearate and starch showed probe binding to be with the hydrocarbon end of stearic acid rather than the carboxyl end. Wide-angle X-ray diffraction patterns were more crystalline as the amylose content of starch was reduced and were typical of each starch type.

In an earlier study (Pearce et al 1985), 16-DOXYL-stearic acid spin probes, but not the more hydrophilic, water-soluble TEMPO spin probes, showed strong binding to starches at room temperature. This difference was attributed to differences in hydrophilic character of the two probes. Calculations based on surface area of starch granules suggested that additional binding sites were needed to account for the observed degree of spin broadening of the probes. The binding of 16-DOXYL-stearic acid to starches persisted after samples were heated and cooled to room temperature. The similarity of spectra before and after heating with subsequent cooling, however, does not answer the question of whether probe mobility changes during the heating cycle.

Therefore in this study, stearic acid-starch interactions were further evaluated at elevated temperatures and compared to spectra recorded before heating and after subsequent cooling. In addition, the probe interaction with starch was compared when the position of the free radical relative to the fatty acid tail was varied, and when the carboxyl group in the fatty acid was replaced by a methyl ester. Starches with different amylose and amylopectin contents were used.

Starch crystallinity patterns prior to electron spin resonance (ESR) studies were verified for each starch by X-ray diffraction.

MATERIALS AND METHODS

Starch Samples

The starches used were wheat starch (Aytex P, General Mills, Inc.), waxy corn starch (Amioca, American Maize Products Co.), and high-amylose (70%) corn starch (Amaizo Amylomaize TM VII, American Maize Products Co.).

Spin Probes

The probes, 16-DOXYL-stearic acid, 5-DOXYL-stearic acid, and methyl 5-DOXYL-stearate were obtained from Aldrich Chemical Co. and used as received. Structures are shown in Figure 1. The probes were dispersed in water as described previously (Pearce et al 1985). Probe-water weight ratios were 1:1,000. All preparations were magnetically stirred for 24 hr prior to use in starch experiments. Solubility of these probes is low, less than 1:1,000, so that a two-phase system resulted.

Preparation of Starch-Water-Probe Samples

Starch, water, and probe samples were prepared by adding starch to the probe-water preparation in starch-to-water weight ratios of 1:2. Each sample was slurried for 24 hr at room temperature before the ESR spectrum was recorded. Samples were transferred to 2-mm glass tubes while slurrying. ESR spectra were recorded at room temperature, elevated temperatures, and after heating and cooling to room temperature.

Heating Studies

Wheat starch, corn starch, high-amylose and waxy corn starches were used with 16-DOXYL-stearic acid probes for these studies. Samples were heated for 4 min at the specified temperature in the ESR spectrometer, which was equipped with a variable temperature controller. ESR spectra were recorded in 10°C increments from 55 to 95°C. ESR spectra were also determined after the samples were cooled to room temperature.

ESR Spectra

ESR spectra were measured and analyzed as described previously (Pearce et al 1985). Measurements were made with a Varian E-3 spectrometer at about 9.33 GHz at either room temperature or elevated temperatures. The spectra were recorded in the vicinity of 3.2 G with attenuation power low enough to avoid any saturation.

Correlation times (τ) were calculated only when a simple three-line spectrum was present. All correlation times were calculated

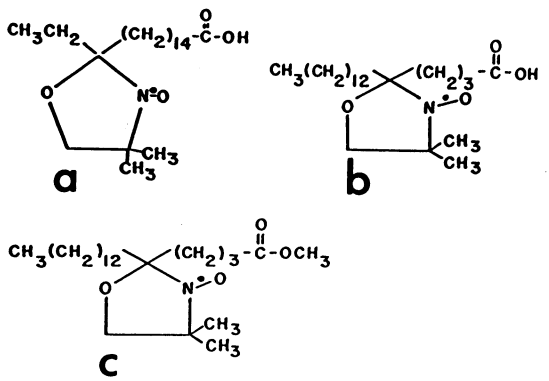

Fig. 1. Structure of spin probes: **a**, 16-DOXYL-stearic acid; **b**, 5-DOXYL-stearic acid; and **c**, methyl 5-DOXYL-stearate.

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assuming isotropic motion of the nitroxide radical and were based on the Kivelson theory (Kivelson 1960). Peak-to-peak line height (h) ratios of first derivative spectra and the line width of the central line $[T_2(0)]^{-1}$ (Stone et al 1965) were used. Thus:

$$\tau = 4 \left[\left(\frac{h(0)}{h(1)} \right)^{1/2} + \left(\frac{h(0)}{h(-1)} \right)^{1/2} - 2 \right] b^{-2} \left[T_2(0) \right]^{-1} \quad (1)$$

Wide-Angle X-Ray Diffraction

Wide-angle X-ray diffraction patterns for each starch were collected using a Siemens D500 diffractometer. Dry starch was loaded at a uniform depth on flat aluminum plates. Samples were scanned from 3 to 33° 2θ, the angle of the incident beam relative to the sample. Relative crystallinity of starch types was qualitatively evaluated by the methods of Nara et al (1978) and as utilized by Dragsdorf and Varriano-Marston (1980).

RESULTS

ESR Spectra of Spin Probes

Typical ESR spectra for 16-DOXYL-stearic acid, 5-DOXYL-stearic acid, and methyl 5-DOXYL-stearate are shown in Figure 2 for the spin probes neat, in a two-phase aqueous system containing dissolved and undissolved probe, and in a saturated aqueous solution where undissolved "chunks" of probe were not included in the sample.

Spectra for neat probes had similar line shapes consisting of one very broad line (Fig. 2a–c). This broad line was the result of spin broadening caused by high spin concentration.

Spectra for the two-phase aqueous systems for each probe (Fig. 2d–f) were demonstrated by one very broad line as in the case of the neat probes, caused by spin broadening in the dispersed spin probe particles. Scan intensity was less than that of the neat probes because of the necessity for smaller sample sizes for aqueous systems.

Spectra for saturated solutions of 16-DOXYL-stearic acid and 5-DOXYL-stearic acid (Fig. 2g and h) showed sharp three-line spectra with $\tau = 1 \times 10^{-10}$ sec and 2×10^{-10} sec, respectively. No ESR signal was obtained for methyl 5-DOXYL-stearate, which indicated that its solubility was less than about $10^{-6} M$.

ESR Spectra of Starch-Water-Probe Systems Measured at Room Temperature Before and After Heating

The spectra of room temperature samples and samples that had been heated and then cooled to room temperature were examined in further detail. Typical spectra are shown for wheat starch-water-16-DOXYL-stearic acid systems measured at room temperature before heating (Fig. 3a) and after heating to 65 or 95° C with subsequent cooling (Fig. 3e and i). These are consistent with spectra reported in the earlier study (Pearce et al 1985). The sharp three-line spectrum of 16-DOXYL-stearic acid in water (Fig 2g) was replaced by a dilute spin, broad-line powder pattern. This powder pattern was present both initially and after heating to 65 or 95° C and subsequent cooling to 22° C. The dilute spin, broad-line powder pattern indicates greatly slowed motion of the probe that is caused by binding or adsorption by stearic acid to starch. Some small changes within the general powder pattern were found, however. For example, the low- and high-field line shapes were slightly different for the unheated room temperature spectrum (Fig. 3a), the sample heated to 65° C (Fig. 3e), and the 95° C sample (Fig. 3i). The outer hyperfine extrema $2A_{ZZ}$ do not change in these spectra but there is indication that a small component of the spins is moving faster after samples were heated.

Spectra for parallel experiments with corn starch, high-amylose corn starch, and waxy corn starch are also shown in Figure 3. As reported earlier, the general characteristics are similar to those for wheat starch in that the broad-line powder pattern is present; however, some differences in probe immobilization were present among the starches. Corn starch and high-amylose corn starch resembled wheat starch closely (Fig. 3b, c, f, g, j, and k), but waxy corn starch (Fig. 3d, h, and l) showed less probe immobilization. In addition, the spectra recorded after heating at 65 and 95° C followed by cooling (Fig. 3h and l) were sharper than those of the unheated system (Fig. 3d), suggesting the probe is more mobile in samples heated to higher temperatures before cooling to room temperature.

ESR Spectra of Starch-Water-Probe Systems at Elevated Temperatures

Representative spectra for starch-16-DOXYL-stearic acid systems are shown in Figure 4 for spectra recorded at 65 and 95° C. Spectra for wheat starch (Fig. 4a and e), corn starch (Fig. 4b and f), and high-amylose corn starch (Fig. 4c and g) at elevated temperatures showed a gradual loss of the powder pattern spectra recorded before heating (Fig. 3a–c). By 95° C, a three-line spectrum was obtained.

Spectra for the two-phase 16-DOXYL-stearic acid and water system at 22 and 95° C are shown in Figure 5. The presence of the small low and high field lines superimposed on the spin-broadened line indicates only a small increase in the solubility of the probe at 95° C as compared to 22° C. This small increase in probe solubility at elevated temperatures (Fig. 5), coupled with the diminution in

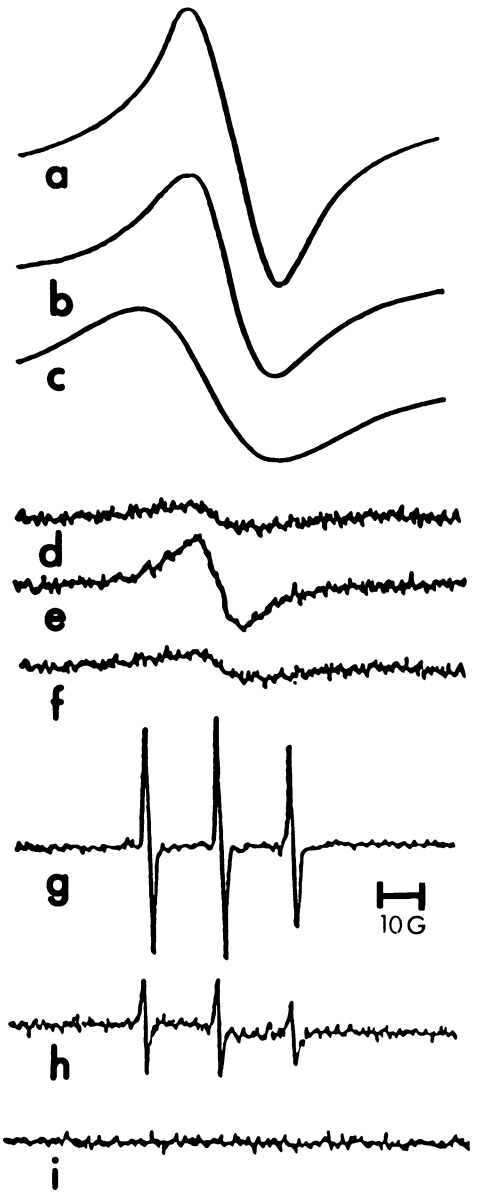

Fig. 2. Electron spin resonance spectra for 16-DOXYL-stearic acid, 5-DOXYL-stearic acid, and methyl 5-DOXYL-stearate: **a–c,** neat; **d–f,** in a two-phase aqueous system containing dissolved and undissolved spin probe; and **g–i,** in a saturated solution.

the intensity of the powder pattern and subsequent development of a strong three-line spectrum in the starch-water-probe system (Fig. 4), suggests that the three-line spectra for these systems at elevated temperatures were caused primarily by the release of bound probe rather than increased solubility of the probe.

Spectra for waxy corn starch-water-probe systems recorded at 65 and 95° C are shown in Figure 4d and h. Generally, the pattern for waxy corn starch is similar to those for amylose-containing starches (wheat, corn, and high-amylose corn starches). However, the gradual loss of the powder pattern spectrum began at lower temperatures for waxy corn starch. The waxy corn starch spectrum recorded at 65° C (Fig. 4d) had a sharper three-line spectrum than the other starches indicating release of the bound probe at lower temperatures.

Wide-Angle X-Ray Diffraction

Wide-angle X-ray diffraction scans are shown in Figure 6. Scans showed similar peak position and intensity, typical of the A type pattern for wheat starch, corn starch, and waxy corn starch (Fig. 6a, b, and d). High-amylose corn starch scans (Fig. 6c) showed less intensity and fewer peaks than other starch types corresponding to the B pattern (Zobel 1964). When X-ray diffraction scans were qualitatively assessed for crystallinity, similar to procedures used by Dragsdorf and Varriano-Marston (1980), the high-amylose corn starch was found to be less crystalline than the other starch types. The more amorphous character displayed by the scan for high-amylose corn starch can be attributed to the high level of amylose (70%) in the starch and consequently decreased amounts of amylopectin, the more crystalline component of starch (Banks and Greenwood 1975).

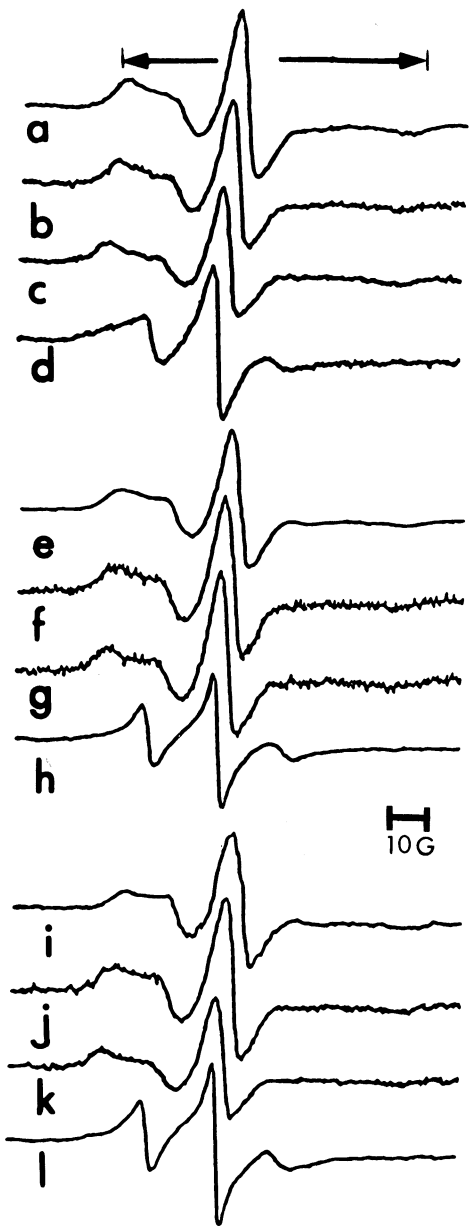

Fig. 3. Electron spin resonance spectra for slurries of starch-water-16-DOXYL-stearic acid. At room temperature: **a,** wheat starch; **b,** corn starch; **c,** high-amylose corn starch; **d,** waxy corn starch. After heating to 65° C and cooling to room temperature: **e,** wheat starch; **f,** corn starch; **g,** high-amylose corn starch; **h,** waxy corn starch. After heating to 95° C and cooling to room temperature: **i,** wheat starch; **j,** corn starch; **k,** high-amylose corn starch; **l,** waxy corn starch. The arrows indicate the position of the outer hyperfine extrema $2A_{ZZ}$.

ESR Spectra with 5-DOXYL-Stearic Acid and Methyl 5-DOXYL-Stearate

Spectra of wheat starch-water-5-DOXYL-stearic acid or methyl 5-DOXYL-stearate are shown in Figure 7 and can be compared with the spectra for the 16-DOXYL-stearic acid probe-starch system (Fig. 3a). All show the broad line powder pattern. In addition, separation of the low field line into a slow and fast component was present in the 5-DOXYL-stearic acid probe system indicating greater probe immobilization (Bobst 1979).

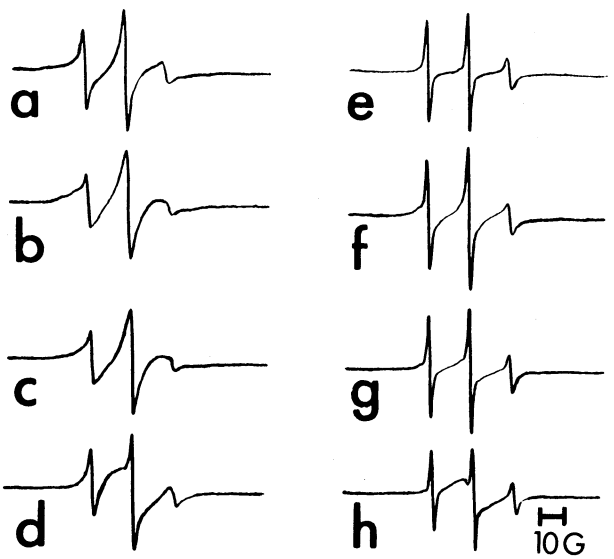

Fig. 4. Electron spin resonance spectra for systems of starch-water-16-DOXYL-stearic acid at elevated temperatures. At 65° C: **a,** wheat starch; **b,** corn starch; **c,** high-amylose corn starch; **d,** waxy corn starch. At 95° C: **e,** wheat starch; **f,** corn starch; **g,** high-amylose corn starch; **h,** waxy corn starch.

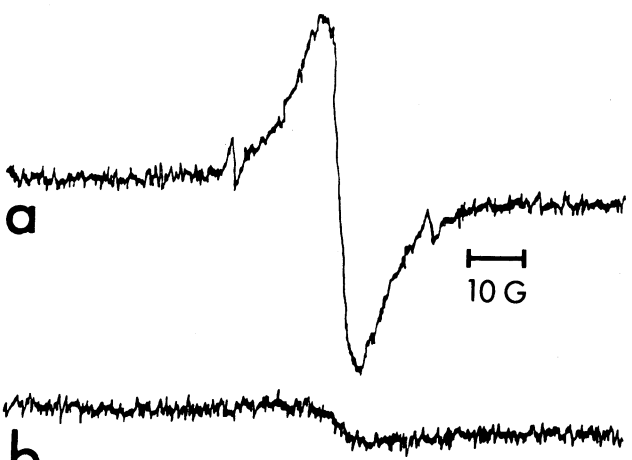

Fig. 5. Electron spin resonance spectra for 16-DOXYL-stearic acid-water containing both dissolved and undissolved spin probe at: **a,** 95° C or **b,** 22° C.

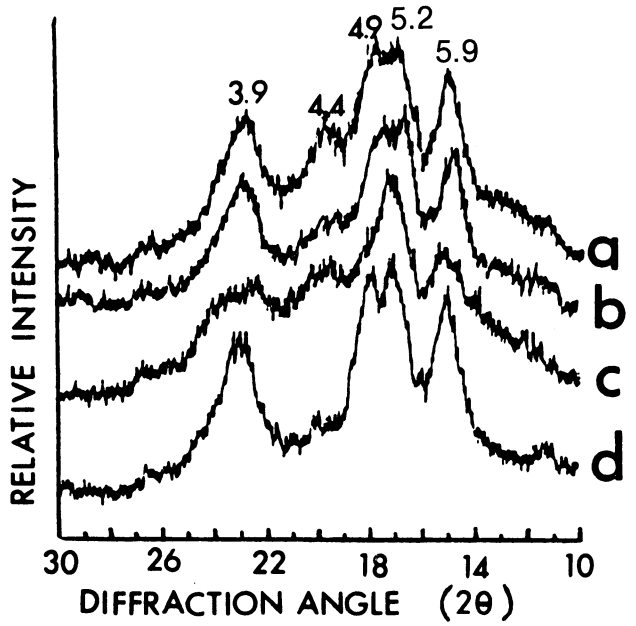

Fig. 6. Wide-angle X-ray scans for: **a,** wheat starch; **b,** corn starch; **c,** high-amylose corn starch; **d,** waxy corn starch. D-spacings are above each peak.

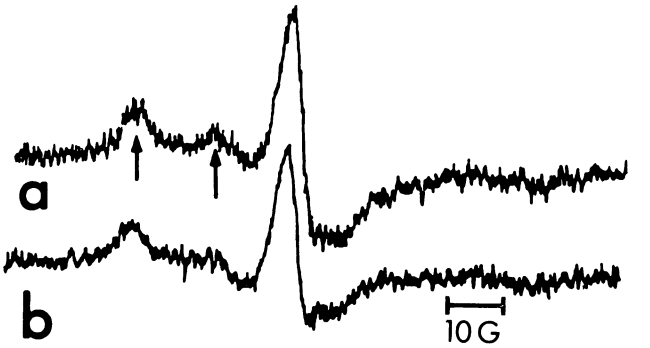

Fig. 7. Electron spin resonance spectra at room temperature for: **a,** wheat starch-water-5-DOXYL-stearic acid or **b,** wheat starch-water-methyl 5-DOXYL-stearate. Arrows on spectrum **a** indicate the slow and fast motion components of the low-field line.

The greater immobilization indicated by this separation may be due to the longer hydrophobic tail, $(CH_2)_{12}$ in the 5-DOXYL derivative versus (CH_2) in the 16-DOXYL derivative. The hydrocarbon tail presumably can probe the hydrophobic environment to a greater depth and thus be immobilized to a greater degree. Miller (1979) showed, by using probes of different functionality, that the immobilization of spin probes is through the functional group rather than the nitroxide moiety.

Further evidence of the role of the hydrocarbon portion of the probe is found in the spectrum of the methyl ester (Fig. 7b). The spectrum of the wheat starch-water-methyl 5-DOXYL-stearate system was identical to that of the free fatty acid probe system.

DISCUSSION

These studies thus far have shown the need for a hydrophobic probe to demonstrate binding of the probe at starch/water ratios of 1:2. Several observations led to this conclusion. First, in our initial study (Pearce et al 1985) probing of aqueous environments of the hydrated starch system with the more hydrophilic probe TEMPO showed slowed probe motion. Slowed motion of TEMPO was a result of binding of water by starch, increased local viscosities within starch granule microcomponents, or a combination of these two effects. Slowed motion of the TEMPO probe was not caused by binding of the probe with starch, because TEMPO was easily removed by successive washings of the starch. Second, the hydrophobic probe, 16-DOXYL-stearic acid, was not removed from the starch by washing (Pearce et al 1985), indicating that stearic acid was strongly adsorbed or bound. The conversion of the ESR line shape to a dilute spin, highly immobilized spin system (Fig. 2 vs. Fig. 3) shows the spin probes to be transferred from and through the aqueous solution to be bound molecularly to the starch. Third, when the length of the hydrocarbon tail was increased from one CH_2 group to 12 CH_2 groups, greater probe immobilization occurred. Fourth, blocking the carboxyl group by esterification did not change the extent of immobilization of the probe, indicating that binding of the stearic acid requires hydrophobic interactions.

The immobilization of probe through the stearic acid occurs at room temperature; the probe is gradually released during heating but is again immobilized during cooling. The original granule structure does not appear to impede penetration of the probe, but neither is the immobilization enhanced by a disrupted granule structure as shown by the similarity of spectra determined at room temperature before and after heating.

The similarity of spectra before and after heating also indicates that bulk viscosity of the system, changes in starch, and probe solubility, which change with heating, are not major factors in the slowed motion. Localized differences, which may be encountered by the probe as it moves from outside the granule to the interior of the granule, may play an important role. Both high-amylose and waxy starches show the powder pattern typical of strongly immobilized probes. The immobilization patterns for both starch types are more similar than would be expected if immobilization were caused only by formation of amylose-fatty acid associations. Nevertheless, there are fine differences in the powder pattern spectra for these starches that may be related to differing granule structures of the starches. Wide-angle X-ray diffraction showed less crystallinity in amylose-containing starches. This diminished crystallinity could facilitate penetration of the probe.

Alternately, both long linear segments of amylose and short linear segments in amylopectin could complex with the stearic acid probe, with the longer amylose segments being more effective than the short segments.

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