The Effect of Fatty Acid Spin Labels Upon Starch[1]

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ABSTRACT

Microscopic studies of waxy corn starch slurried with TEMPO-laurate, 16-DOXYL stearic acid, or TEMPO-benzoate showed that all three probes caused gelatinization of the starch at room temperature. Regular corn and wheat starch were not affected by the probes.

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During a study of native and gelatinized starch systems by electron spin resonance (ESR), anomalous behavior was observed when waxy corn starch was mixed with fatty acid spin labels (Nolan et al 1986). Intermolecular bonding is weaker in waxy starch than in normal starch, presumably because of the absence of amylose (Banks et al 1970). This causes waxy starch to swell readily and produce a high peak viscosity, but the granular structure then collapses and the viscosity decreases. Gelatinization of waxy starch involves granule swelling, loss of birefringence, and dispersion of granular remnants (Gough and Pybus 1971). Previous reports utilizing waxy starch and probe (Pearce et al 1985) did not report any anomalous behavior.

The purpose of this study was to examine the interaction of TEMPO-laurate, 16-DOXYL stearic acid, and TEMPO-benzoate with wheat, corn, and waxy corn starches.

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MATERIALS AND METHODS

Materials

Starch. A commercial, unmodified wheat starch was obtained from Midwest Solvent Co. (Atchison, KS). Corn starch was obtained from the CPC Co. (Argo, IL), and waxy corn starch from Clodualdo Maningat (Kansas State University).

Spin labels. 16-DOXYL stearic acid was obtained from Aldrich Chemical Co. (Milwaukee, WI). TEMPO-laurate and TEMPO-benzoate were purchased from Molecular Probes (Junction City, OR). Figure 1 shows the structures of these molecules. All other reagents were reagent grade.

Sample Preparation

TEMPO-laurate, TEMPO-benzoate, and 16-DOXYL stearic acid were diluted to 2×10^{-5} *M* in ether and added to wheat, corn, and waxy corn starch. The ether was allowed to evaporate, and water was added to achieve a ratio of 2:1:0.002 for water, starch, and probe.

Separation of starch from the solvent was achieved by centrifugation with a benchtop centrifuge for 30 min at 300 rcf. Samples for microscopic examination were taken from the supernatant, top of pellet, and bottom of pellet.

Microscopy. Bright field and polarized images of the same fields were examined with a Zeiss Universal microscope equipped with

Olympus objectives. Photographs were taken using Kodak Tri-X Pan film.

RESULTS AND DISCUSSION

In the presence of TEMPO-laurate, 16-DOXYL stearic acid, and TEMPO-benzoate (Fig. 1A–C), waxy corn starch formed a viscous solution. Neither wheat starch nor regular corn starch exhibited this phenomenon. In addition, the ESR spectra (Fig. 4C, Nolan et al 1986) from waxy corn starch and TEMPO-laurate in water were not as distorted as those from wheat starch and regular corn starch. Clearly, the interaction of this probe with waxy corn starch was different than with the other starches.

When either 16-DOXYL stearic acid or TEMPO-laurate were added to waxy corn starch, the granular appearance was altered radically. The supernatants after centrifugation remained cloudy. Bright field microscopy of the supernatant showed that it contained a large number of granular remnants, fragments, and starch granules in various stages of disintegration (Fig. 2A).

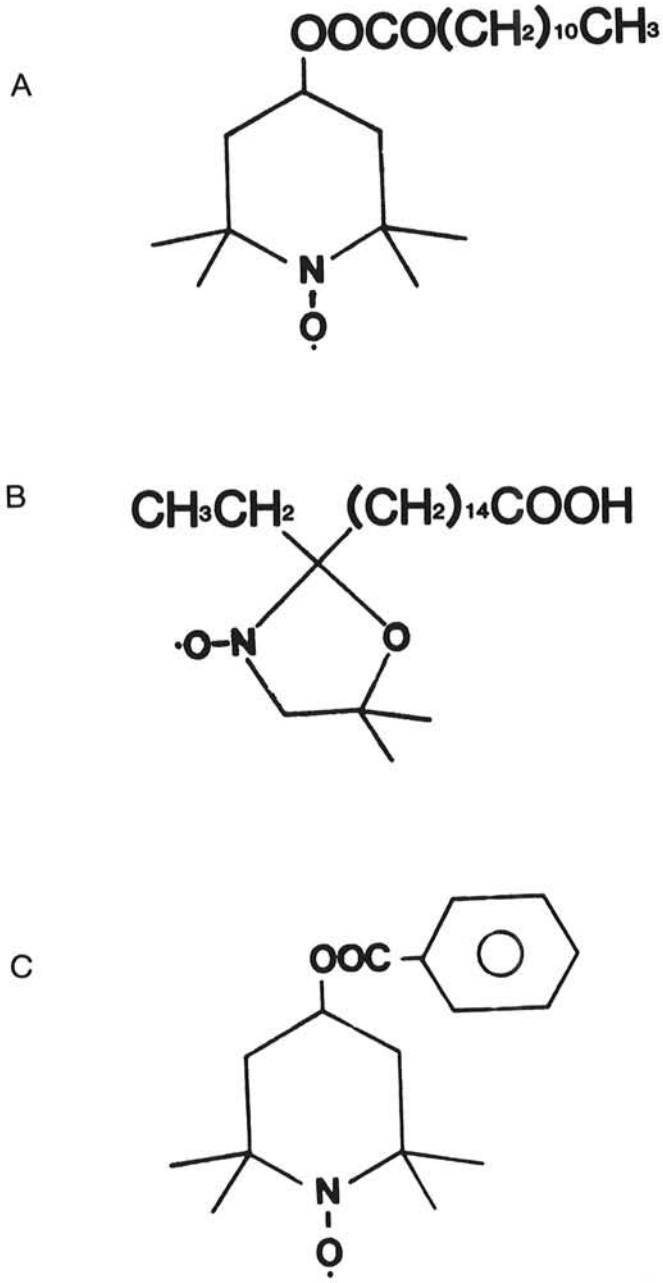

Fig. 1. The chemical structure of (**A**) TEMPO-laurate, (**B**) 16-DOXYL stearic acid, and (**C**) TEMPO-benzoate.

Examination with crossed Nicols showed that few, if any, of the granules retained birefringence (Fig. 2B).

Examination of the last material to be deposited as sediment, the top of the starch pellet, revealed similar, yet less severe, changes in

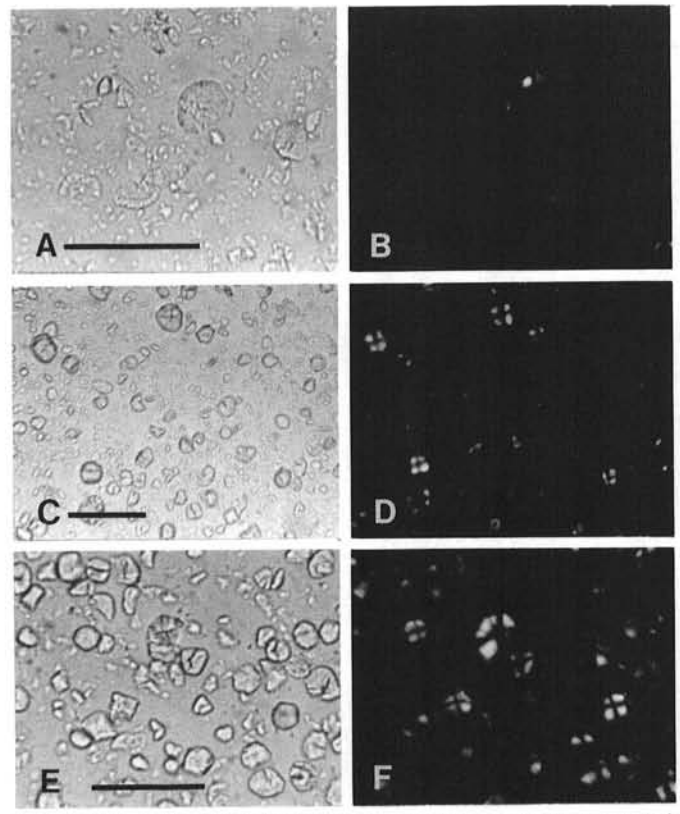

Fig. 2. Bright-field and polarization photomicrographs of waxy corn starch treated with TEMPO-laurate: (**A**) and (**B**) supernatant, (**C**) and (**D**) top of pellet, and (**E**) and (**F**) bottom third of pellet. Scale bars equal 100 μm.

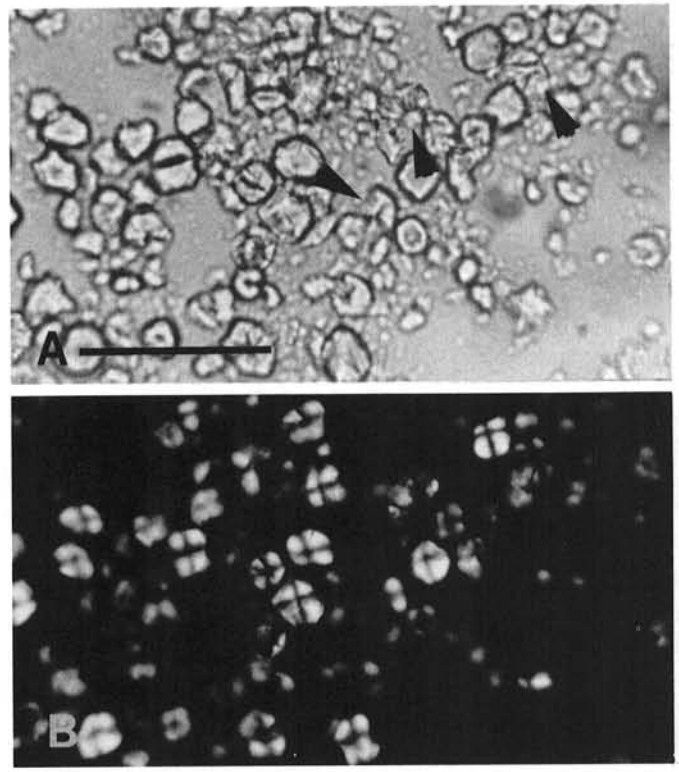

Fig. 3. Bright-field (**A**) and polarization (**B**) photomicrograph of waxy corn starch with TEMPO-laurate, no centrifugation. The starch sample was kept in suspension. Scale bars equal 100 μm.

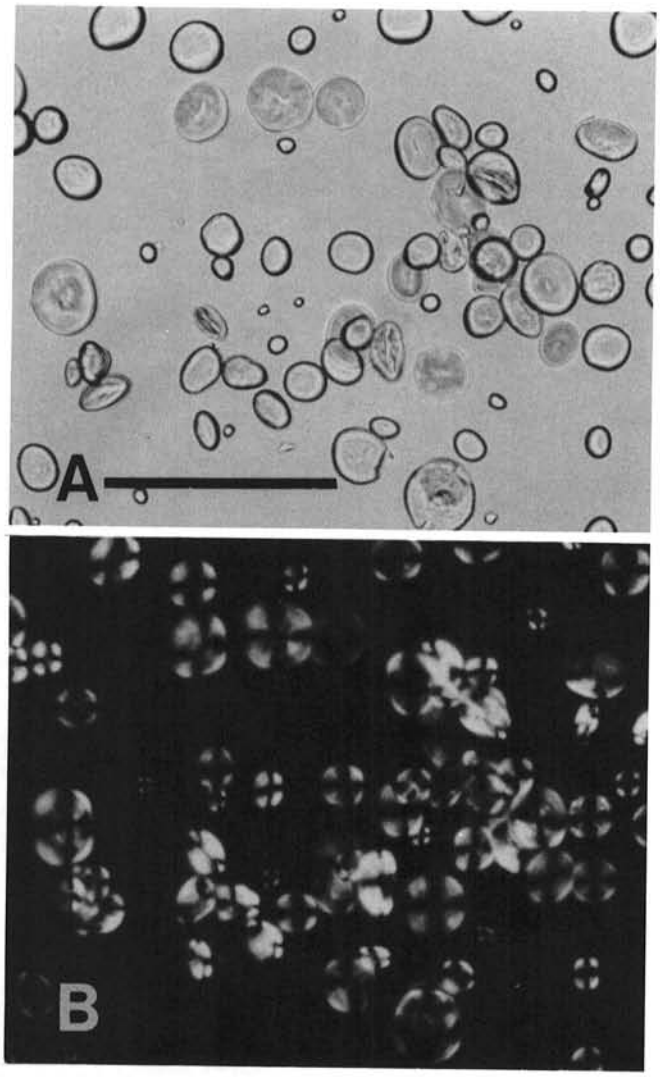

Fig. 4. Bright-field (**A**) and polarization (**B**) photomicrograph of wheat starch with TEMPO-laurate. Scale bars equal 100 μm.

the starch (Fig. 2C and D) compared to the supernatant. Here, granular remnants were conspicuous, as were granules in the process of disintegration. However, 10–20% of the granules, as well as some of the larger fragments, retained birefringence. Starch from the bottom third of the pellet, the first to settle, was also disrupted (Fig. 2E and F), but the disruption was less severe than at the top of the pellet.

The pattern of granular disruption was quite unexpected. The granules were not exposed to significant amounts of heat at any time during the experiment. In addition, the presence of fatty acid has not been reported to cause changes in granular structure. Still, the changes observed (radial disintegration, loss of birefringence) are symptomatic of gelatinization of waxy corn starch. Arrows in Figure 2 indicate where this unique type of disintegration occurred.

The presence of a fatty acid-linked spin label apparently destabilized the waxy corn starch granule to such an extent that gelatinization occured at room temperature. This phenomenon is not without precedent, because dimethylsulfoxide (Me_2SO) will cause granule disintegration at room temperature (Leach and Schoch 1962). However, the difference in concentration is striking (90% for Me_2SO versus 10^{-5} M for the fatty acid probes). The probes described here are at least qualitatively similar to the amino oxides that were shown to be solvents for cellulose (Johnson 1985).

The possibility remained that, although spin label-starch interaction took place, the granule disintegration or disruption occurred because of the stress encountered during centrifugation. To test this, waxy corn starch plus TEMPO-laurate was allowed to stand without centrifugation. Samples from the resuspended

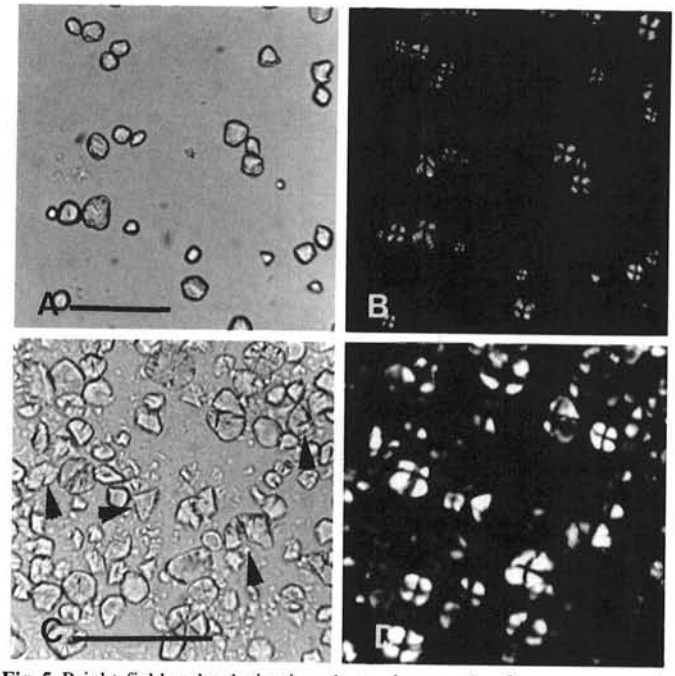

Fig. 5. Bright-field and polarization photomicrographs of waxy corn starch and TEMPO-benzoate: (**A**) and (**B**) supernatant, (**C**) and (**D**) pellet. Scale bars equal 100 μm.

starch (Fig. 3) clearly showed that granule disintegration took place without centrifugation.

Wheat starch, which pelleted cleanly in studies designed to take ESR spectra, did not change in its microscopic appearance because of interaction with TEMPO-laurate (Fig. 4). Granules were neither swollen nor fragmented. They all retained their birefringence. This does not rule out the possibility that some sort of destabilizing interaction may have taken place. For this starch, the changes may not be manifested at room temperature.

TEMPO-benzoate also caused disruption of waxy starch granules, but the granule disintegration and loss of birefringence were much less severe than those found with TEMPO-benzoate (Fig. 5). The same types of changes were still evident, however. Figure 5, for example, shows granular remnants and granules in the process of coming apart radially (arrows), as well as intact granules. It appears that the probe-starch interaction that results in destabilization is not caused only by the fatty acid substituent on 16-DOXYL stearic acid and TEMPO-laurate, but is mediated by both the spin label and fatty acid component. The severity or, perhaps more accurately, the extent of the destabilization appears to be affected by the type of hydrophobic moiety.

A probe's interaction with the host is a clear violation of a spin label's requirement to remain unreactive in a system. However, because the radical's signal is preserved, ESR analysis is still possible. The probes clearly generate changes in waxy starch. More study of this phenomenon is warranted before the use of 16-DOXYL stearic acid and TEMPO-laurate in starch systems can be clearly understood.

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