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HETEROGENEOUS REACTION OF GRANULAR STARCH WITH HYDROGEN CHLORIDE

I. Microscopic Observations¹

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ABSTRACT

A heterogeneous reaction between anhydrous granular starch and hydrogen chloride gas takes place at 70°C. The reaction is characterized by an induction period of 2 to 17 hr., depending on the starch used and the HCl pressure, after which there is a sudden evolution of water. The color of the starch darkens during the induction period and turns black soon thereafter. Some degradation occurs during this period. Microscopic and hydration studies on the products indicate that disorganization, with attendant cold-water solubility, increases to a maximum during the induction period. A sudden relaxation of the granule seems to occur at the end of the induction period, allowing dehydration to begin. The subsequent products become insoluble again, apparently through some sort of cross-linking, and the color becomes completely and permanently black. Motion-picture studies of the hydration behavior of the induction period products revealed some of the granular aspects of the reaction.

The granular structure of starch is of considerable importance in reactions utilizing starch in this form. It is also likely that a study of the granule might shed some light on the molecular structure of starch. The granule varies in shape and size over large ranges, depending on the source of the starch. Much has already been published on microscopic and hydration studies of the granule (1,2,3,4). It is here proposed that considerable information regarding the granule, possibly useful in characterizing starches, can be revealed through heterogeneous reactions between the dried starch and either gases or liquids in which the starch does not swell. The kinetics of such reactions particularly reflects the structure and condition of the starch and is the subject of the second paper in this series. The present paper deals with the microscopic aspects of the reaction of granular starch with gaseous hydrogen chloride.

Previous studies by Barham and one of the authors (5,6) involving the reaction of starch with liquid chlorine revealed some kinetics that were definitely related to granule structure. For example, an induction period of about 8 hr. was noted when corn starch reacted at 70°C. A year later the induction period for this same starch was found to have increased to more than 12 hr. This was attributed to "aging" of the starch. Pretreated, disorganized starches

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in which the gross granular structure was destroyed had greatly reduced induction periods. The zero-order (with respect to starch) shape of the curve during the induction period suggested that the rate of reaction was limited to the rate of diffusion of chlorine into the granule. Accordingly, it was reasoned that the main factor controlling this rate was the hydrogen-bonded structure of the starch. This was confirmed by the fact that catalytic amounts of water added to the starch-chlorine mixture to serve as a hydrogen-bond breaker successfully reduced the induction period in proportion to the amount used.

It was felt that hydrogen chloride, a by-product of chlorination, might be the hydrogen-bond breaker or disorganizer of the granule in the chlorine reaction, since it was unlikely that chlorine could accomplish this. Pretreatment of starch with anhydrous hydrogen chloride was then found to substantially reduce the induction period during chlorination. During this pretreatment the starch changed from white to black, though retaining its granular structure. Upon chlorination of this black product a bright-yellow starch dichloride was formed, similar to that obtained with untreated starch. This indicated that the pretreatment had not appreciably changed the starch chemically but rather had disorganized it. The nature of the blackness was not apparent. Investigation of induction period products in the chlorination reaction revealed that a black intermediate was also formed during this stage, again suggesting that HCl was involved. It therefore seemed worthwhile to investigate the HCl reaction by itself to see what information it might yield concerning the structure of starch and the starch granule.

It should be pointed out that the reaction described here is not to be confused with previously reported acid treatments of starch for the purpose of its modification and degradation into dextrins. These are normally carried out under other than anhydrous conditions and with catalytic amounts of acid.

Materials

All starches were used in their natural condition, to preserve granule structure. None were extracted, and all were dried in an Abderhalden drying apparatus under vacuum for 2 hr. while being heated with boiling toluene at 110°C. The potato starches used here were obtained from the Idaho Potato Starch Company in Blackfoot, Idaho. The corn, wheat, and rice starches were obtained from ordinary commercial sources. The hydrogen chloride was regular grade from Matheson Chemical (> 99.0% pure) and was used without further purification.

Methods

Reaction with Hydrogen Chloride. The reaction is effected by placing about 10 g. of dried starch into a 200-ml. Erlenmeyer flask connected with ground joints to a three-way junction. One arm of the latter is connected to a 1-liter flask⁴ filled with HCl gas, the second is connected via stopcock to a

⁴ It was later found that similar results could be obtained when less than 1 g. of starch and correspondingly smaller volumes of HCl were used, provided the same ratio and initial pressure were maintained.

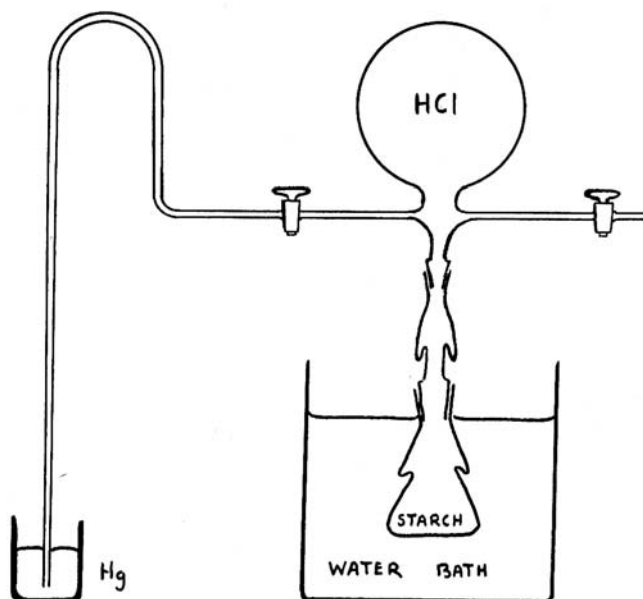


Fig. 1. Apparatus for reaction of starch with hydrogen chloride gas.

mercury manometer, and the third leads to another stopcock through which HCl is introduced or the system is evacuated. The flask is immersed in a constant-temperature bath, the system is evacuated, and HCl is introduced by means of a rubber balloon. The system is normally filled to atmospheric pressure at the beginning of the reaction. Figure 1 is a schematic representation of the apparatus.

Photomicrography. All pictures, both still and motion shots, were taken through a Spencer, student-grade monocular microscope. The still shots were taken on 35-mm. Kodak Plus-X film for black and white pictures, and on Kodachrome II, type "A," and Anscochrome T-100 for the color pictures. A Pentax single-lens reflex camera was used with an ASAHI adapter by removing both the lens from the camera and the eyepiece lens from the microscope. Images were focused on the ground glass of the camera before exposure was made with a cable release.

Motion-Picture Studies. A Bolex 16-mm. motion-picture camera was used, with Tri-X high-speed black and white negative film subsequently developed in a D-76 developer. The pictures were shot directly through the camera lens immediately above the microscope eyepiece. Focusing was very near to that for direct vision, and was checked by the reflex viewing device of the Bolex camera. The lens was set wide open and exposures were controlled by a voltage regulator for the substage light, according to the number of frames per sec. taken, the filters used, and the magnification. "Slow-motion" pictures were obtained by shooting at 64 frames per sec. The starch pictures showing hydration were obtained by placing the dry products under a cover glass and

adding small droplets of water by dropper while the camera was running. The water flowed by capillarity into the field of the camera.

Electron Micrographs. Electron microscope studies were made on the University's Philips EM-100B instrument. Direct observations of granules were made by sprinkling granules on a Formvar support film on 200-mesh copper grids. Surface details were obtained by means of plastic impressions of the granules made with acetyl cellulose film in a two-stage replica process using palladium-gold shadowing at 30° backed with carbon.

Results and Discussion

Physical Appearance of Products. As the reaction proceeds, the most obvious change is color. The starch slowly darkens over a period of 10 hr. or longer at 70°C., depending upon the conditions of the run. The products go through a sequence of colors best described as pink, tan, malt, cocoa, chocolate, dark brown, and finally black. The rate of color formation seems to be independent of the amount or kind of starch used in a given apparatus as long as the initial pressure of HCl is kept constant and an excess of HCl maintained. However, in a series of runs made with the same starch with various pressures of HCl, it was found that the lower the HCl pressure, the slower the colors developed. Comparison of different starches, such as rice, corn, wheat, and potato, indicated that the colors for these developed at the same rate under the same conditions.

Temperature change has the expected effect on the rate of color formation. Raising the temperature to 80°C. approximately doubled the rate at 70°C. At room temperature the reaction eventually takes place, but about 10 days' time is required to reach the same stage as is reached in 10 hr. at 70°C. Throughout these color changes, the starch retains its original texture.

The one other noticeable feature of the reaction is the delayed evolution of moisture. After some time has elapsed, drops of condensate appear on the cooler part of the apparatus, just above the water level of the bath. The time varies from as early as 3 hr. for rice starch to as late as 17 hr. for potato starch; the moisture continues to form for a number of hours and then gradually diminishes. The initial evolution of water is closely related to some other properties, one of which is the behavior of the products to water. The products at this stage of the reaction (where water first appears) are at their maximum disorganization, and swell rapidly in cold water. A continuous record of the weight of the product shows a maximum just before this point, with subsequent decrease in weight as water is lost. The amount of the product that is extractable in cold water is also at maximum here. Finally, there is a sudden change in the rate at which HCl is absorbed. All these quantitative aspects, which are presented in Part II of this series, indicate that something critical happens at this time, corresponding closely to the behavior noted in the liquid-phase chlorination of starch, where it was termed an induction period; the same term is applied here and will be used hereafter to indicate this phase of the reaction.

It was first thought that the end of the induction period was the point where the product became black as this was the case when potato starch was

reacted with HCl at 70°C. However, other starches, particularly under different conditions, showed this not to be true in general, and the appearance of water is taken as a better indication of the end of the induction period. Later work showed that an even better estimate of the induction period can be obtained by following the pressure of HCl in the system, as there is a sudden change in the rate of pressure at this point.

One further difference that seems to occur with regard to color is that prior to the end of the induction period the colors are not stable, but change on standing, particularly if exposed to air. Hydration studies indicate that the color is removed completely by water up to the end of the induction period, whereas after this point the color seems to become fixed and, in fact, the products become water-insoluble. The early products (meaning before the end of the induction period) also change color on heating, generally lightening or turning reddish, whereas the later products remain permanently black.

Microscopic Observations. Samples were taken at regular intervals during the reaction of potato starch with HCl at 55°C. This temperature was chosen, rather than 70°C, to facilitate the resolving of stages of the reaction. The samples were observed microscopically under plain and polarized light.

The effect of water on the products was most enlightening. Water was added dropwise in such manner that it could be seen making contact with the granules as it spread by capillary action under the cover glass. The resulting action, in some cases, was so rapid that it could not all be caught with the eye. To study it better and to record it, motion pictures were taken at various speeds.⁵

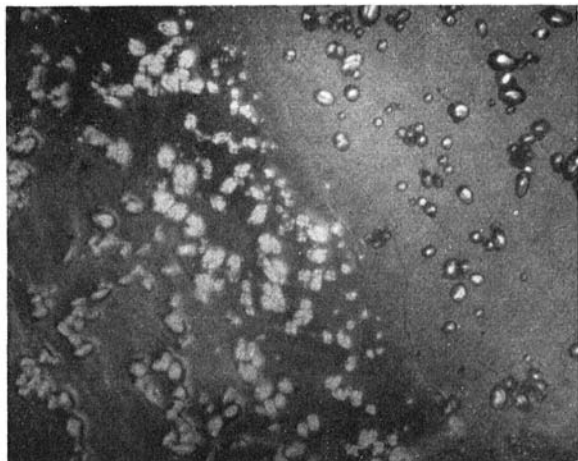


Fig. 2. Effect of water on early induction period product, polarized light. Note improvement of interference pattern.

Under polarizing light, typical interference crosses were seen in decreasing intensity up to the black stage, where they disappear. (The term *black stage* will be taken to mean the *first* formation of a black product, marking the end

⁵ The photographic studies were made by Dale Petite.

of the induction period in this case; the products remained black thereafter.) As with unreacted starch, the interference pattern was more visible when water was added (see Fig. 2). The smaller granules lost this pattern earlier than the large ones.



Fig. 3. Effect of water on early induction period product. Note that granules turn white with water at this stage.

About halfway to the black stage, where the product was malt-colored, small radial cracks began to appear in the granule. These increased up to the black stage where observation became difficult owing to the opacity of the product.

In all stages up to the black stage the granules turned white as water touched them (Fig. 3). Material could be seen being extracted. It was quite apparent that only a portion of the granule had been affected by the HCl, in proportion to the time reacted. With samples less than halfway to the black stage, the outer reacted portion swelled rapidly and began to dissolve. This portion became isotropic to polarized light while the inner, unreacted portion still showed polarization crosses as sharp as those of the original starch and swelled at a much slower rate. The hilum was very pronounced in the intact portion to the last (Fig. 4, a-e). The extracted material had the appearance of a brown syrup and dried to a varnishlike film that cracked on further drying. It was obvious that the longer the reaction, the more material was extracted, until at the black stage the product appeared totally soluble in cold water.

Just prior to the black stage a membrane appeared to form that was not previously noticeable. The granules swelled rapidly with water in less than a second's time giving the appearance of inflated balloons. The swelling appears to be a combination of diffusion of water into the granule through this membrane, aided by visible osmotic effects; possible degradation and sub-

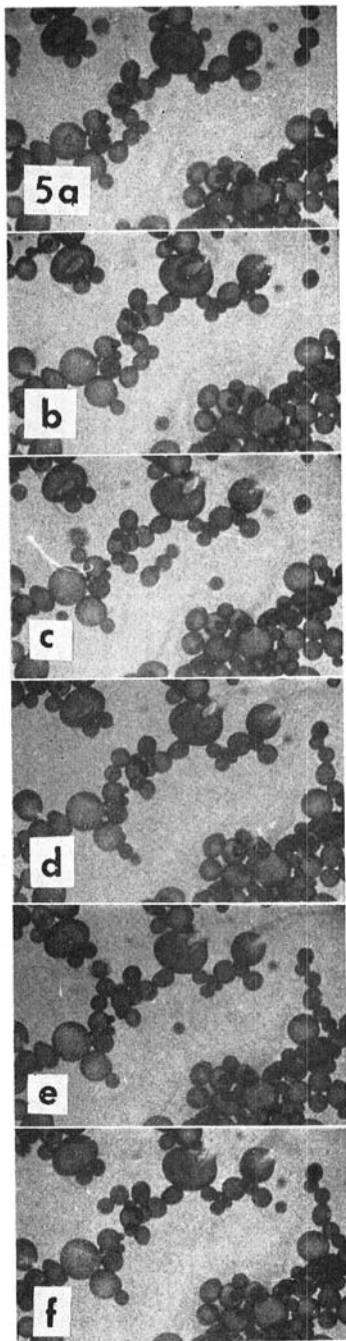
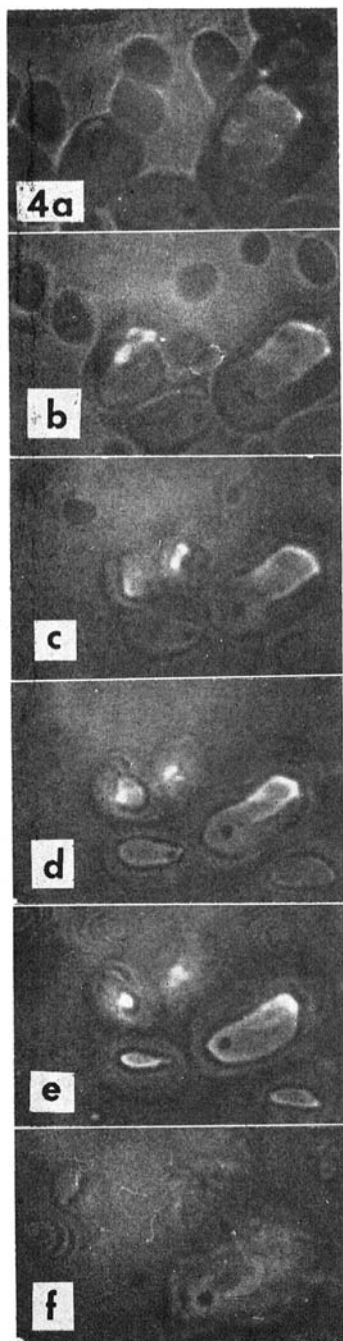


Fig. 4 (left), a-f: Effect of water on early induction period product; $\frac{1}{8}$, $\frac{3}{8}$, $\frac{5}{8}$, $\frac{7}{8}$, $\frac{9}{8}$, and $\frac{25}{8}$ sec. resp. after water added.

Fig. 5 (right), a-f: Effect of water on black-stage product; frames $\frac{1}{4}$ sec. apart after water added. Note mixed swollen and polymerized granules. Also note loose inner granules escaping from ruptured granules.

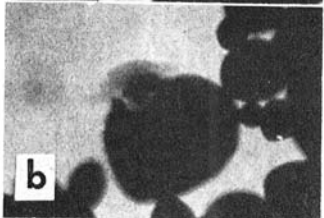
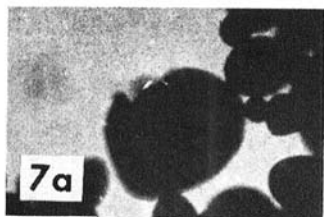
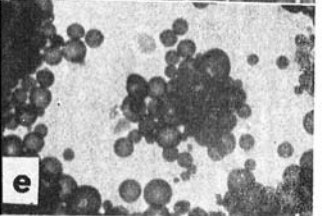
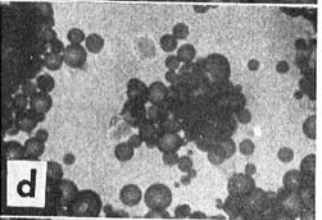
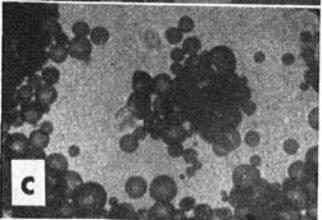
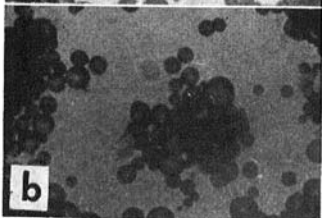
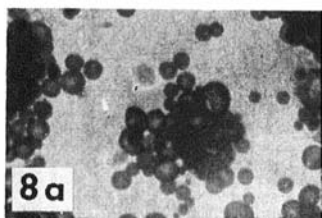
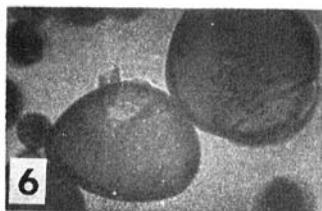


Fig. 6 (upper left). Ruptured black-stage granule after treatment with water, showing membrane.

Fig. 7 (lower left), a-c: Effect of water on black-stage product; three consecutive frames at 24 frames/sec. after water added. Note expulsion of inner granule by osmotic explosion.

Fig. 8 (right), a-e: Effect of water on black-stage product. Five consecutive frames at 64 frames/sec. after water added. Note osmotic rupture of granule near left center. Also note that smaller granules are past the induction period and no longer swell.

sequent solubilization are indicated. In some instances (Fig. 5, a-f) a residual inner granule, representing the unreacted portion of the starch, could be seen

to rotate relative to the membrane, showing the liquid consistency of the material inside the swollen granule. The resulting osmotic pressure often resulted in rupture of the membrane, which was clearly visible in many granules (Fig. 6) and was caught in the process of happening with the motion-picture camera (Fig. 7, a-c). The elastic nature of this membrane was evident in Fig. 8, a-e, where a protrusion was observed whose length approximated half the diameter of the granule. This elongated protrusion then ruptured, and extracted material was seen streaming out.

Just at the black stage, swelling proceeded with almost explosive speed the moment the granule came in contact with water. No inner granule remained, and the swollen granule invariably disintegrated into fragments. Owing to distribution of sizes of the granule, it was also apparent that the larger granules had not quite reached this stage, whereas the smaller ones showed evidence of having already passed this point and having entered the "inert" stage described below.

After the black stage the amount of swelling with water decreased pro-

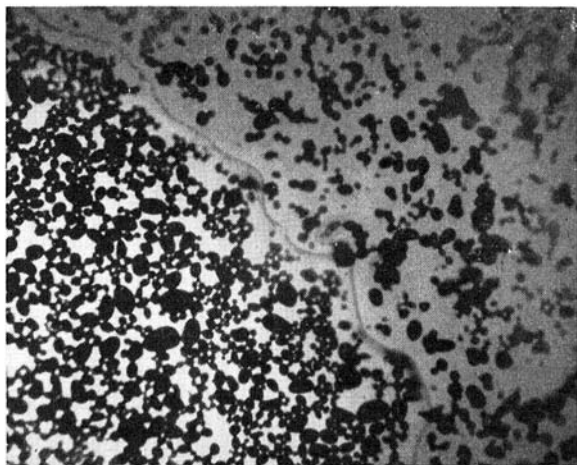


Fig. 9. Effect of water on product past induction period. Note loss of swelling and extraction, and retention of color.

gressively until at about five times the induction period, swelling ceased entirely and the products were inert to water. All extractability disappeared and the products remained jet black and opaque; they also had a shiny surface, and a hard, gritty texture when rubbed between glass slides. Upon contact with water the granules appeared slightly charged and tended to repel one another, congregating only because of surface tension (Fig. 9). Within a minute after the addition of water, a peculiar erratic motion was observed as an occasional granule suddenly propelled itself. The source of this motion was seen to be the emergence of a small bubble of gas (Fig. 10) that had worked its way out of the granule, possibly through a crack, and moved the granule in an opposite direction. This effect was found in all the later products of the

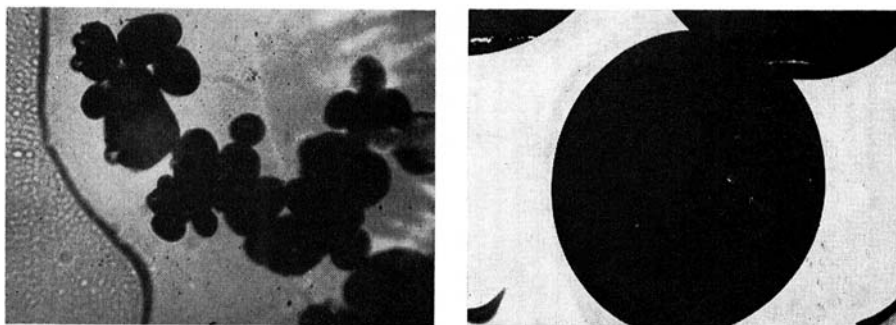


Fig. 10 (left). Effect of water on product far past induction period. Note small gas bubbles emerging from granules.

Fig. 11 (right). Electron-microscope silhouette of polymerized product.

reaction. Mechanical handling, such as grinding or sieving, of these dry products invariably resulted in the development of electrical charge.

Electron-Microscope Studies. Comparisons were made between starch and the various products with the electron microscope.⁶ Direct viewing of starch granules caught on a plastic grid was not useful; they proved opaque and the beam quickly affected and changed the granules. The black products proved to be much more resistant to the beam than raw starch, and good silhouettes, though no details, could be obtained. These electron micrographs indicated that the reacted granules were very smooth and round (Fig. 11).

Plastic impressions of the granules yielded good detail of surface structure of these products. Figure 12 is an electron micrograph of the replica of a 70°C., 138 hr. product, well beyond the induction period, made from potato starch. It shows a definite, nonperfect, hexagonal packing of structures throughout the surface of the granule. The granule shown is about 15 μ in diameter. The large, hexagonally packed structures are about 3 μ across and contain 14 or so helical-shaped bundles whose helix diameter appears to be about 0.2 μ . Some of these appear to be intertwined or braided into a cable about 0.4 μ in diameter. Each bundle appears to be made of smaller helical structures about 0.015 μ in diameter, which also make up the matrix of unorganized matter for the entire granule. These smaller units of 0.015 μ or 150 A diameter, which appear to be the limit of resolution in this picture, agree reasonably well with the "microfibrils" reported in Linternized potato starch (7,8). These microfibrils are about two orders of magnitude larger than the eventual starch molecule helix. If we assume the seven-strand structure postulated by Sterling and Pangborn, the microfibril would be composed of micellar strands of about 50 A, in this case. These strands, in turn, could well consist of seven-strand cables of amylose chains, whose diameter would then be of the order of 15-17 A, in accord with crystallographic measurements for the amylose helix. Since each successive stage of helix twists necessarily in an opposite direction,

⁶ The electron micrographs were taken by James R. Swafford, electron microscope technician at the Department of Botany, Arizona State University.

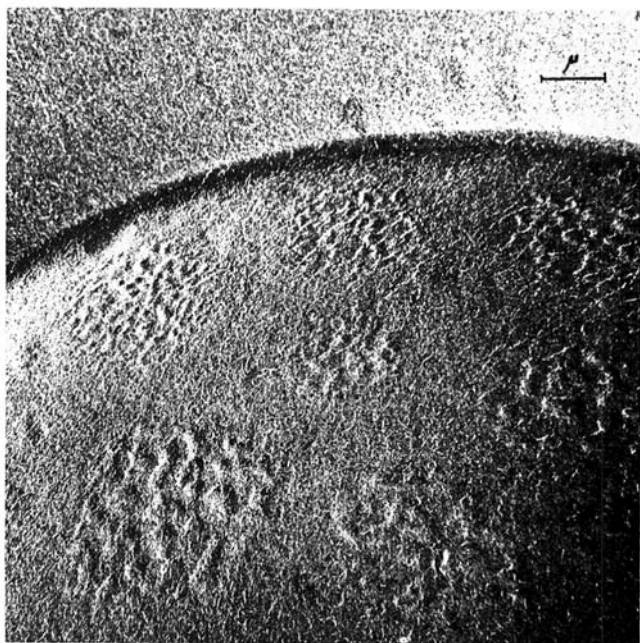


Fig. 12. Electron micrograph of palladium-carbon-shadowed impression of polymerized product. Note hexagonal packing of bundles.

the right-hand structure of the visible bundle would require a left-hand twist for the microfibril, a right-hand twist for the micelle, and a left-hand twist for the amylose helix; this is also in accord with current belief.

Similar electron micrographs for unreacted starch did not yield as good detail as these reacted products. Apparently the treatment with HCl, in some way, brings out the microfibrillar structure.

General Discussion and Conclusions

From the qualitative work described here, several things are apparent about the reaction of granular starch with hydrogen chloride gas. There is a dehydration reaction that occurs only after a pronounced induction period, the length of which is different for various starches. Disorganization of the granule occurs during the induction period, reaching a maximum at the end of the period. The starch at this point is rapid-swelling and soluble in cold water. There is little doubt that the induction period represents a diffusion of hydrogen chloride into the granule, as intermediate products show partial penetration and partial disorganization in proportion to the fraction of the induction period involved.

After the induction period, the dehydration reaction apparently involves some sort of polymerization, possibly cross-linking, of the starch, as there is a subsequent decrease in solubility of the starch until the final product is hard,

inert, and insoluble in water. The black color, which was extractable up to the end of the induction period, becomes permanent and unextractable. The nature of this black color is unknown.

The initial reaction of hydrogen chloride appears to be one of disorganization as the HCl diffuses into the granule. Presumably, the hydrogen chloride breaks intermolecular hydrogen bonds between hydroxyl groups and oxygen atoms in neighboring glucose units of different amylose chains. Penetration is radial, as evidenced by the hydration behavior of the partially disorganized products. The rate of this initial disorganization reaction is definitely related to the pressure of the hydrogen chloride, as the induction period was approximately doubled when the pressure of HCl was halved.

The fact that dehydration apparently does not occur until after the induction period would seem to indicate that some sort of over-all relaxation occurs when the HCl reaches the center of the granule. This relaxation allows the various helical structures in the starch the freedom to move close enough to permit chemical interaction between groups on separate molecules that heretofore were unable to meet.

The differences in induction period noted for different starches indicate that this reaction might serve to characterize starches. Much of this is related to granule size, as the smaller-sized starches have shorter induction periods, but undoubtedly the degree of organization also is involved. A quantitative study of the kinetics of this reaction seemed worth while and is reported in a following paper.

Electron-microscope studies show that the subgranular structure of starch is brought out by the reaction with HCl. Evidence is seen for hexagonal packing of structures that contain as many as 14 bundles of smaller helical structures, in turn made up of numerous still smaller helices. The latter appear to be the microfibrils previously reported by earlier workers.

Acknowledgment

The authors wish to gratefully acknowledge the early study of this reaction by workers in this laboratory (9). The support of the National Science Foundation, making possible summer work by high-school students Charles Cowley and Sandra Weber, is greatly appreciated, as is the contribution made by these students. An NSF Academic Year Institute has made possible the work by the junior author (D. P.). An NSF Faculty Research Grant program has allowed the senior author (T. R. T.) to spend some of his time on this work during the summers of 1963 and 1965. The electron microscopy by James R. Swafford is also gratefully acknowledged.

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