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Gel structure and water diffusion phenomena in starch gels studied by pulsed field gradient stimulated echo NMR

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Water diffusion phenomena in potato starch gels (starch concentrations in the range of 17.1–42.0 w/w%) were observed as restricted diffusion by the pulsed field gradient stimulated echo (PFGSTE) NMR method. The size of water compartments, a, permeability of the barriers, p, and self diffusion coefficient of compartmentalized water between the barriers, D_0, were estimated using a theoretical equation. These parameters changed with both starch concentration and duration of storage time. The structure of the starch gels and the structural change during retrogradation are discussed with respect to these parameters.

INTRODUCTION

Almost all foods, including starch are prepared by heating with water to change starch into paste or gel states. It is well known that the rheological nature of the starch gels in foods affects the properties and quality of the foods and is strongly dependent on gel microstructure. It is also known that starch gels change their rheological nature during storage at room temperature or low temperature (i.e., retrogradation). In many cases, retrogradation causes undesirable textual properties in starch-based foods. Therefore, the microstructure of such gels is one of the most important factors controlling the properties of starch-based food materials. In order to study gel structure and its change during retrogradation, a non-destructive *in situ* investigation is necessary. For this purpose, X-ray diffraction, small angle X-ray scattering (SAXS), DSC and NMR have been applied.

X-ray diffraction and SAXS can provide the information about recrystallization and aggregation of polymers (Miles *et al.*, 1985; Matsukura *et al.*, 1983; Orford *et al.*, 1987; Cameron & Donald, 1991), but they cannot examine molecular dynamics. Although qualitative changes in molecular dynamics associated with phase transitions can be detected by DSC, DSC is not suitable for quantitatively measuring molecular dynamics and gel structure.

NMR has been used widely to investigate the molecular dynamics and the water binding behavior of the gel matrix as well as their changes during retrogradation (Lechert, 1981; Mola-Gutierrez & Baiana, 1989; Blanshard *et al.*, 1990). Almost all of these investigations have been carried out by using high-resolution NMR in both liquid and solid states and by observing proton relaxation phenomena. In addition, recently, the changes in dynamics of the solid-like component of the starch gel have been investigated using cross-relaxation analysis (Wu *et al.*, 1992; Wu & Eads, 1993). Pulsed field gradient NMR is also a promising method for investigating the microstructure of the starch gel on the basis of the diffusional behavior of water.

The diffusional phenomena of compartmentalized water, such as water in biological cells, reflect the size and shape of the microstructure of the compartments and permeability of the barriers (Tanner & Stejskal, 1968). When water mobility is restricted by the barriers, one observes an apparent diffusion coefficient decreasing with increasing diffusion time. The pulsed field gradient (PFG) NMR method (Stejskal & Tanner, 1965; Tanner, 1970) is suitable for observing such restricted diffusion. In this method, localized diffusional phenomena during short diffusion times ranging from 10 to 1000 ms are observed. Von Basler and Lechert (1974) and Callaghan *et al.* (1983) observed the diffusion of water in corn starch gels and in various wheat starch pastes, respectively, by using the PFG spin echo-NMR method. In neither case was restricted diffusion observed. Von Basler and Lechert measured the signal attenuation due to diffusion for only short diffusion times with a maximum of 50 ms, which is too short to detect the restricted diffusion. Callaghan *et al.* sampled

dilute pastes with starch content of up to 10 w/w%. In the present paper, diffusion of water in rather concentrated potato starch gels (17–42 w/w%) is investigated by the pulsed field gradient stimulated echo (PFGSTE) NMR method (Tanner, 1970) for a very wide range (60–1010 ms) of diffusion times. The structure of fresh starch gels and the structural changes in the gel during retrogradation are discussed.

MATERIALS AND METHODS

Sample preparation

Potato starch was obtained from Wako Pure Chemical Industries, Ltd, Tokyo, Japan. The water content in the potato starch used was determined to be 16% by the air oven method (A. O. A. C. Method, Horwitz, 1980). We included this water in calculating the solvent concentration of starch gels.

In order to prepare homogeneous highly concentrated samples, the apparatus shown in Fig. 1 was hand-made. It mainly consists of a sample rotor and a water bath. An NMR sample tube (an outer diameter = 5 mm) containing a starch–water mixture was horizontally inserted into the water bath from the bottom. The temperature of the sample was kept at 98.7±0.4°C. With this apparatus, we could heat the starch–water systems while adequately mixing them by rotating the sample tubes. The rotating rate was about 60 rpm.

Potato starch granules were weighed directly into an NMR sample tube. Distilled water was added to the tube to make the suspension. The suspensions were held at room temperature for 3 days; then, they were heated and kept at 98.7±0.4°C for 1 h with mixing by using the sample rotor mentioned above. The samples were cooled down at room temperature for about 20 min prior to the NMR measurement. These fresh starch gel samples had concentrations in the range from 17.1 to 42.0 w/w%. All fresh samples were stored at 5°C for retrogradation after NMR measurement. For convenience, samples under investigation were labeled as S_{ij} as shown in Table 1, where i represents the concentration of starch and j represents the duration of storage at 5°C.

Measurement of diffusion coefficient

All measurements were carried out by using MSL100 Spectrometer (Bruker) at 23°C. The pulsed field gradient stimulated echo (PFGSTE) pulse sequence (Tanner, 1970) shown in Fig. 2 was used. Symbols δ, Δ, and g are the duration of field gradient pulse [ms], the duration for diffusional motion (diffusion time) (ms), and the amplitude of the applied field gradient pulse (mT/cm), respectively, as indicated in Fig. 2. τ_1 and τ_2 are the duration between the first and the second $\pi/2$ pulses and between the first and the third $\pi/2$ pulses, respectively. The parameters were set as follows: the value of g was changed from 0.5 to 5.0 mT/cm at each 0.5 mT/cm, and the value of Δ was arranged in the range of 60–1010 ms. The values of τ_1 and δ were fixed as 10 ms and 1 ms, respectively. The length of the $\pi/2$ pulse was determined for water proton in each sample and was in the range of 8.9–9.4 μs. The accumulation numbers of the scans were 16 for sample S_{1j} (concentration 17.1 w/w%) and 8 for other samples. Only the stimulated echo was accumulated. The signal intensity of the water proton was obtained from the spectrum by Fourier transformation of the stimulated echos. Spectral width was 5000 Hz or 10 000 Hz.

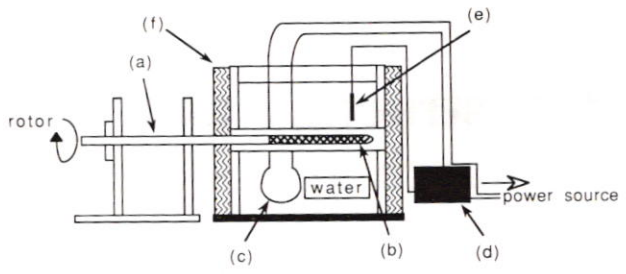

Fig. 1. An apparatus for making starch gel samples; (a) sample tube, (b) sample, (c) heater, (d) thermostat, (e) sensor, and (f) thermal insulating material.

Table 1. The preparation conditions of starch gel samples

Concentration	Storage time at 5°C				
	0 day	1 day	2 days	3 days	4 days
17·1%	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}
27·3%	S_{20}	S_{21}	S_{22}	S_{23}	S_{24}
33·3%	S_{30}	S_{31}	S_{32}	S_{33}	S_{34}
37·9%	S_{40}	S_{41}	S_{42}	S_{43}	S_{44}
42·0%	S_{50}	S_{51}	S_{52}	S_{53}	S_{54}

In this pulse sequence, the ratio of the echo amplitude obtained in the presence of the field gradient to the echo amplitude in zero field gradient, R, is expressed as

$$\ln(R) = -D(\gamma\delta g)^2\left(\Delta - \frac{\delta}{3}\right), \quad (1)$$

under an assumption that the relaxation and the diffusion are simply factorizable. For free water diffusing within an infinitely large, homogeneous space, a self diffusion coefficient, D, is obtained from the slope of a graph of $\ln(R)$ vs $(g\delta)^2(\Delta - \delta/3)$ plot. On the other hand, the diffusion coefficient obtained from the slope changes with the diffusion time, Δ, in the case of the restricted diffusion. Therefore, the $\ln(R)$ vs $(g\delta)^2(\Delta - \delta/3)$ plot results in straight lines with different slopes for each Δ value. The diffusion coefficients obtained from the slopes are considered an apparent diffusion coefficient, D_{ap}.

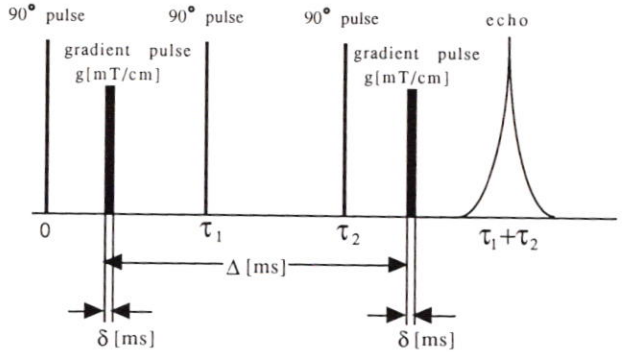

Fig. 2. Pulsed field gradient stimulated echo pulse sequence.

The dependence of the stimulated echo amplitude on T_1 and T_2 is described by Stilbs (1987):

$$A(\tau_1 + \tau_2) = 0{\cdot}5\,A(0)\exp\{-(\tau_2 - \tau_1)/T_1 - 2\tau_1/T_2 - D(\gamma g\delta)^2(\Delta - \delta/3)\}. \quad (2)$$

The left side in eqn (1) is the ratio of the echo amplitudes with and without field gradient. Therefore, the effect of T_1 and T_2 relaxations on the echo amplitudes (eqn (2)) is neglected in eqn (1) under the assumption mentioned above. A cross relaxation between water proton and polymer proton may be occurring during the longitudinal storage period and affects the T_1 relaxation time (Wu *et al.*, 1993). Usually, the intra- and inter-molecular cross relaxation times in such systems are in the order of 1–10 s (Akasaka *et al.*, 1990; Era *et al.*, 1989). The cross relaxation effect is therefore assumed to be negligible when we use a diffusion time of less than a second. Moreover, the effect of the cross relaxation is mostly cancelled as well, because the cross relaxation is similarly effective to T_1 without distinction of the presence or the absence of the field gradient.

RESULTS

Figure 3 shows the relationship between $\ln(R)$ and $(g\delta)^2(\Delta-\delta/3)$ in the case of S_{50}. When Δ and δ are kept constant and g is changed in a stepwise manner, a series of $\ln(R)$ should be linear to $(g\delta)^2(\Delta-\delta/3)$. The observed data showed a good linearity for each Δ value in the range of 60–610 ms. The data indicate that there exists only one type of diffusion component in the starch gel system under the diffusing conditions investigated so far.

The apparent diffusion coefficient, D_{ap}, sequentially decreases with increasing Δ, which means that diffusional motion of water in the starch gels is restricted by the network of the starch gels. All other samples showed identical results. The Δ value dependence of D_{ap} is shown in Fig. 4 with simulated lines based upon an analysis discussed in the next section.

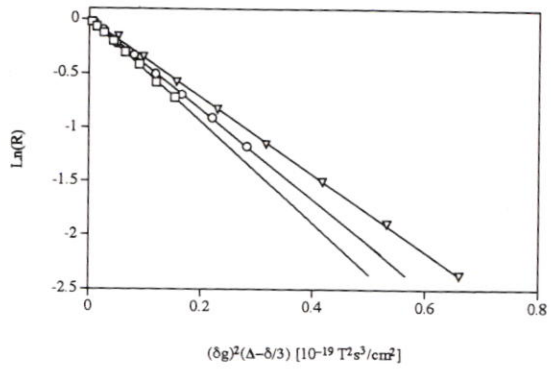

Fig. 3. Relationship between $\ln(R)$ and $(g\delta)^2(\Delta - \delta/3)$ for the sample S_{50}. $\Delta = (\square)$ 60, $(\circ)$ 110 and (∇) 610 ms.

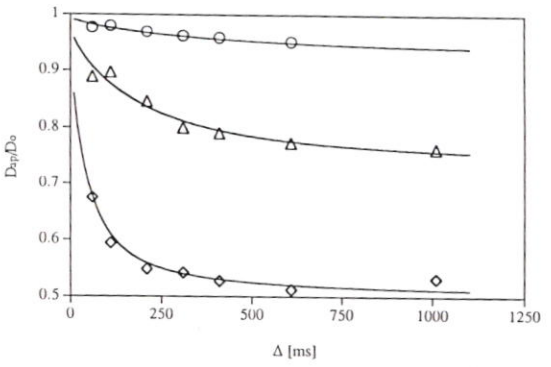

Fig. 4. Relationship between D_{ap}/D_0 and Δ in fresh gels at various concentrations. Symbols show the experimental data for S_{10} $(\circ)$, S_{30} $(\triangle)$ and S_{50} $(\diamond)$. Solid lines correspond to the calculated curve from eqn (3) in the text.

DISCUSSION

Restricted diffusion of water in starch gels

Restricted diffusion of water was observed in starch gels with high concentrations of starch (17.1%–42.0%). The differences in D_{ap} for each Δ value were significant in the range of $0.1–0.3 \times 10^{-5}$ cm^2/s, because the precision of observed D_{ap} was $\pm 0.01 \times 10^{-5}$ cm^2/s as an average standard deviation in our experiments. At first glance, our observations seem to differ from the previous ones by Callaghan *et al.* (1983), who concluded that the starch gel matrix does not form a barrier against the free diffusion of water in the gels. It is important to note, however, the differences in concentration of starch. The starch concentrations in Callaghan's sample were less than 10 w/w%, while those in the present work were in the range from 17.1 to 42.0 w/w%. As shown in Fig. 4, restriction of diffusional motion becomes greater with increasing concentrations of starch. The results of Callaghan *et al.* are in the same line with the present results and indicate that water is more freely mobile in soft pastes than in hard gels. The same tendency was observed in dextran sols and gels (Watanabe & Ohtsuka, 1993).

As the structure of starch gel is too complex to be exactly expressed, we analyzed the restricted diffusional motion by assuming Tanner's model, in which permeable barriers are parallel to each other with the same distance (Tanner, 1978). Diffusion phenomena in such geometry were described by von Meerwall and Ferguson (1981) in eqn (3):

$$R(t) = \exp\left[-\frac{\theta^2 D_0 t}{a^2}(\sin^2\alpha + A)\right]\frac{2}{\pi^2 d^2} \left\{1 - \cos\pi d + 2\sum_{n=1}^{m}\left[\frac{1-(-1)^n\cos\pi d}{(1-n^2/d^2)^2} \exp\left(-\frac{n^2\pi^2 D_0 B t}{a^2}\right)\right]\right\}, \quad (3)$$

where t is the diffusion time, $\theta = \gamma g\,\delta a$, $d = (\theta/\pi)|\cos\alpha|$, a, distance between barriers, D_0, the interbarrier diffusion coefficient, and α, the angle between the barrier and the direction of the principal axis of the field gradient. The reduced permeability, P is expressed as $P = ap/D_0$, where p is the permeability of the barriers. The coefficients A and B correspond to $\cos^2\alpha/(1 + 1/P)$ and $1/(1 + P)$, respectively.

The values of a, p, and D_0 were obtained by fitting eqn (3) to the observed data. Figure 4 shows the result of fitting samples, S_{10}, S_{30} and S_{50} on the same graph, where the ratios of D_{ap} to D_0 were plotted against the diffusion time. The curve calculated from eqn (3) shows a good agreement with the observed values.

The structure of starch gel

In Fig. 5a, b and c, the starch concentrations and aging dependence of D_0, p and a are depicted, respectively. First, with fresh gels (see ◇ mark in Fig. 5), the value of D_0 gradually decreased with increasing starch concentration and was close to a constant value of about 10^{-5} cm^2/s (Fig. 5a). If the diffusional motion of water is caused only by restricted diffusion due to a structural barrier, the interbarrier self-diffusion coefficients D_0 should be equal to the self-diffusion coefficient of pure water in all cases. However, the estimated D_0 values are about half that of pure water. Our observation suggests that D_0 is affected by 'obstruction and hydration effects' due to polymers and parts of polymers which are dispersed in the water compartments (Wang, 1954). In other words, in gels with higher starch concentrations, compartmentalized water is not pure water, but includes some quantity of polymers and/or parts of polymers, with the result that the compartmentalized water experiences higher viscosity and/or a longer lifetime in the hydration sphere of starch chains.

The permeability, p, also sequentially decreased with increasing starch concentration and finally reached a constant value in starch gels at over 30% concentration (Fig. 5b). This result indicates that the network of barriers becomes more dense in gels with higher starch concentrations and that the maximum density of such barriers is limited. This result agrees with the general observation that the higher the starch concentration the stiffer the corresponding starch gel (Orford *et al.*, 1987).

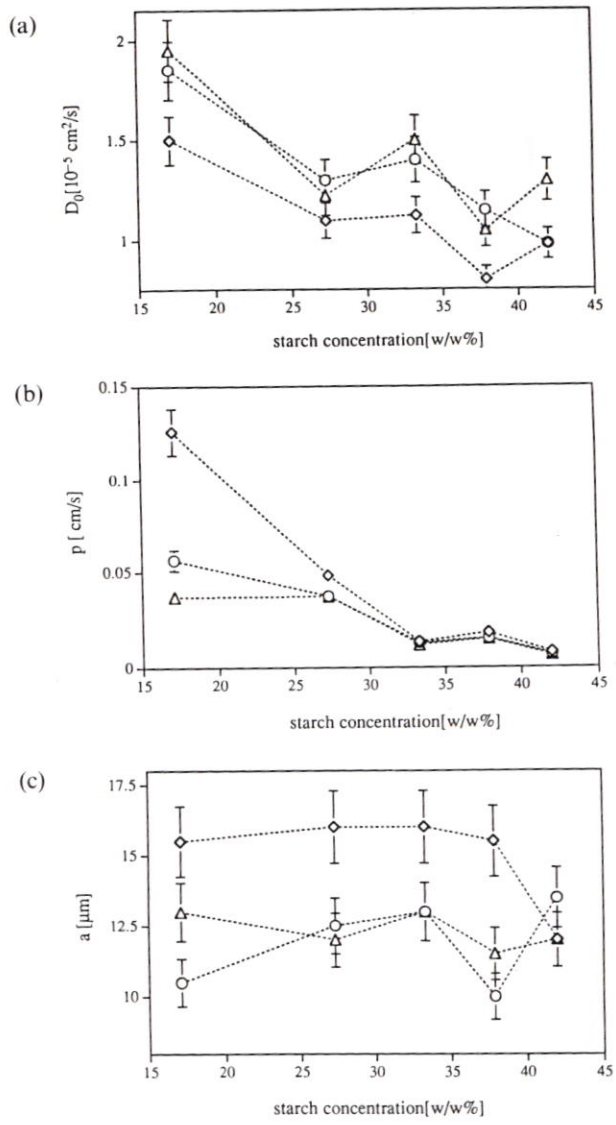

Fig. 5. Concentration dependence of (a) self diffusion coefficient of water D_0, (b) permeability p and (c) size of microstructure a in fresh gels (◇) and gels stored at 5°C for 1 day (○) and 4 days (△). The dotted lines follow the calculated data points in the same series for eye convenience.

The value of the distance between the neighboring barriers, a, is identical (about 15 μm) and independent of the starch concentration in all fresh gels except for the sample S_{50} (see Fig. 5c). Only in the case of this highest concentration, does the distance a become smaller, 80% of the constant value. Note that all aging samples also reach this value (about 12 μm). The increase in polymer concentration causes an increase in the density of barriers until the latter reaches its limit value. When the density of barriers reaches the limit

value (at a starch concentration between 33.3 and 37.9%), further increase in the starch concentration causes a decrease in the a value because the number of the barriers increases.

We can picture the fresh starch gel structure as follows. Some of the polymers aggregate to make the skeletal network of the gel. The remaining polymers, and/or parts of polymer chain, are free from the skeletal network and function as a solute in water compartmentalized by the network. The latter polymers contribute to obstruction and hydration effects. In fresh gels with higher starch concentrations, the network becomes denser and thicker, and the compartmentalized water also includes more polymer chain free from the network. According to results examining the dynamics of the solid-like component of starch gels using cross relaxation (Wu *et al.*, 1992), the dynamics of this component become inert with increasing starch concentration. This means that starch gel becomes stiffer when it is highly concentrated. The structural model mentioned above agrees with the results from the cross relaxation study. It has been shown by X-ray diffraction that the gel matrix is mainly constructed of amylose (Miles *et al.*, 1985). Thus, it also seems likely that the polymers existing in the water compartments are mainly amylopectin.

Changes in gel structure due to retrogradation

As shown in Fig. 5a, the value of D_0 gradually increased with storage time in all cases, indicating that the bulk water in the compartments becomes more mobile. It was shown by high resolution ^{1}H NMR that the dispersed component of starchy polymers in the gel decreases with increasing storage time (Wu & Eads, 1993). Thus it can be reasonably supposed that the amount of dispersed polymer components decreases, resulting in an increasing value of D_0 with storage time.

Figure 5b portrays the relationship between p and storage time. In the sample S_{1j} (17.1 w/w%), the value of p remarkably decreased during one day; and in the sample S_{2j} (27.3 w/w%), the value of p changed only slightly during storage in comparison to S_{1j}. The value of p is independent of storage time in more concentrated gels ($\geqslant$33.3 w/w%). These facts suggest that the density changes in the barrier in retrogradation depend on the concentration of polymer; the thickness or structure of the skeletal converges into the same state. The inter-barrier distance a decreases and reaches nearly the same value in all samples during storage (see Fig. 5c).

X-ray diffraction and SAXS studies have indicated that recrystallization of polymers takes place during starch retrogradation (Miles *et al.*, 1985; Matsukura *et al.*, 1983; Cameron & Donald, 1991). On the basis of this concept, our observations during retrogradation can be explained as follows: the recrystallized polymers contribute to the density of barriers in the dilute samples S_{1j} and S_{2j}; with increasing starch concentration, the recrystallized polymers contribute mainly to construction of new barriers.

Therefore, we can conclude that the structural changes in starch gels during retrogradation are due to the fact that the dispersed component of the polymers in the compartmentalized water gradually aggregates with the original barriers of fresh starch gels; the polymers recrystallize to make the barrier thicker and/or denser. In highly concentrated gels, the polymers in the water compartments apparently recrystallize *in situ* with the close neighboring polymer during retrogradation, because such polymers do not have much freedom in diffusional motion. As a result, the new barriers and/or the rigid aggregations of the recrystallized polymers construct water compartments. In both cases the amount of the soluble component of starchy polymers decreases. This phenomenon leads to an increase in the self diffusion coefficient, D_0, of water in the compartments.

It is generally known that retrogradation of starch gels leads to more rigid gels (Miles *et al.*, 1985; Morris, 1990). It has also been found by X-ray diffraction that recrystallization of amylopectin takes place as a result of retrogradation, which causes stiffness to develop (Miles *et al.*, 1985). In addition, the cross relaxation study by Wu *et al.* (1992, 1993) indicates that the mobility of the solid-like component slows during retrogradation. If we combine these studies with our results, we can conclude that, in the case of relatively dilute starch gels, the gel matrix becomes dense as a result of recrystallization of polymers, which causes development of stiffness. In the case of concentrated starch gels, recrystallized polymers make new barriers and/or lead to larger barrier surface areas. Thus the stiffness of starch gels increases during retrogradation.

CONCLUSION

The diffusion of water in starch gels was found to be restricted. By analysing the data with the model of von Meerwall and Ferguson (1981) we could estimate the permeability of the skeletal structure of starch gels (p), the average size of water compartments (a), and the diffusion coefficient of the compartmentalized water (D_0). These parameters contain valuable information about the structure of starch gels.

The average compartment size of fresh gels other than 42.0% was identical (about 15.5 μm), and did not depend on the starch concentration. An increase in the starch concentration contributes to making the skeletal structure of gel matrix more dense and less permeable. A further increase in starch concentration causes new barriers to grow and the compartmental size decreases to about 12 ± 1 μm.

In the process of retrogradation, the skeletal structure

grows into a more limited structure with identical properties of density and permeability regardless of the starch concentration. The structural change starts immediately after fresh gel formation and mostly occurs within 1 day. The dispersed polymers in water compartments are dehydrated and recrystallize as a result of retrogradation. In relatively dilute gel, recrystallizing polymers aggregate to the original network of the fresh gel. In concentrated gels, polymers recrystallize in the water compartment and occasionally make new barriers in the compartments. The density of the barriers eventually reaches a maximum value as a result of the recrystallization process.

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