Scanning Electron Microscopy and Atomic Force Microscopy of Fibrous Gelatin Observed between AgBr Microcrystals

Hiroshi Saijo*†, Yutaka Kobayashi and Makoto Shiojiri*‡

Department of Electronics and Information Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606 Japan

Gelatin has been the only circumstance for silver halides to grow of photographic use. However structural analyses of gelatin adsorption on silver halide microcrystals have rarely been made with microscopic methods. The present study shows the adsorption of gelatin on AgBr microcrystals with scanning electron or atomic force micrographs taken during the removal process of gelatin in photographic emulsions. Gelatin thin layers remained covering AgBr particles after most of the gelatin matrix was washed out in large quantities of warm water with strong agitation. The thin gelatin layers were again dissolved away and narrow strands of gelatin remained only where AgBr particles were packed close. The diameters of gelatin strands decreased gradually from the end where the strands adsorb on the surface of AgBr to reach the thinnest at the center. The smallest diameter observed in this study was about 20 nm, which must be a strand of many collagen helices. Two different methods to remove gelatin (conventional enzyme decomposition followed by centrifugal separation or the present warm water dilution with agitation and decantation) were compared and the warm water provided better exposed surfaces of AgBr with little gelatin residue.

Journal of Imaging Science and Technology 41: 531–535 (1997)

Introduction

Silver halide microcrystals for photographic use grow only in gelatin sol. Gelatin adsorbs on the surface of silver halide microcrystals, and the gelatin matrix is believed to protect the particles from directly touching each other during the ripening process. Gelatin is made of the natural polymer collagen and has complex high-order structures. The high-order structure is relatively thick, and a scanning ion microprobe could image such a structure by detecting O^- ions from gelatin.[1] The diameter of fibrous materials was about 0.2 to 0.3 μm from the image. No low-order structure of fibrous material in gelatin was observed in the study.

With the rapid progress of scanning probe microscopy, scientists hoped to study the ion arrangements of silver halide microcrystal surfaces including the reconstruction on (111), but no similarities existed between the atomic images of silver halide (111) surfaces. A study concluded the disordered surface of microcrystals grown in gelatin was covered by a thin gelatin layer.[2] After scribing out the disordered surface layer, the particles revealed bulk-exposed surfaces of (100) or (111) arrangement. However, no image of the disordered surface nor any evidence of gelatin was shown in the work. Direct observation of the gelatin layer adsorbed on AgBr tabular microcrystals was made using atomic force microscopy (AFM).[3a,3b] The outer surfaces of gelatin were rough with deep holes of a diameter of 10 to 100 nm reaching down to the AgBr surface. No conclusions were reached about why such big and deep holes existed in the gelatin layer.

Though gelatin is a matrix of fibrous collagen helix, it is usually imaged as a huge mass under electron microscopes with little texture, and the fibrous structure of gelatin is rarely imaged except in our previous study by scanning electron microscopy (SEM).[4] In that study we observed thin gelatin films that connected AgBr particles. A loop of 20-nm-diameter fiber was seen on the surface of AgBr, and we believe the filament to be a fragment of gelatin. The collagen itself has been studied from a biochemical perspective with SEM[5] and AFM.[6a,6b] However, those studies did not lead to any structure for gelatin adsorbed on silver halides.

When studying the surfaces of photographic materials with electron microscopy, electron spectroscopy, and various microscopic techniques, gelatin must be removed in advance of the observation. The gelatin is usually decomposed by enzyme and dissolved away. By this method, most gelatins are washed out and some small fragments remain on the surface. The silver halide particles after the gelatin removal treatment coalesce with surrounding thin gelatin films.[4] Another method to remove gelatin from the photographic emulsion is to wash out the gelatin with warm water.[7] This method excludes condensation during the centrifugal separation usually included in the conventional enzyme fragmentation process and provides a two-dimensional monolayer arrangement of silver halide particles.

The present study shows the shape of the residual gelatin on AgBr cubic crystals after the washout treatment in comparison with our previous work using the enzyme removal process.[7]

Experimental

AgBr cubic microcrystals, 0.7 μm in size, were grown in gelatin following the usual manner in photographic

Original manuscript received February 15th, 1997.

* IS&T Member

† Fax: +81-(0)75-724-7400, e-mail: hsaijo@dj.kit.ac.jp

‡ Fax: +81-(0)75-723-2048, e-mail: shiojiri@dj.kit.ac.jp

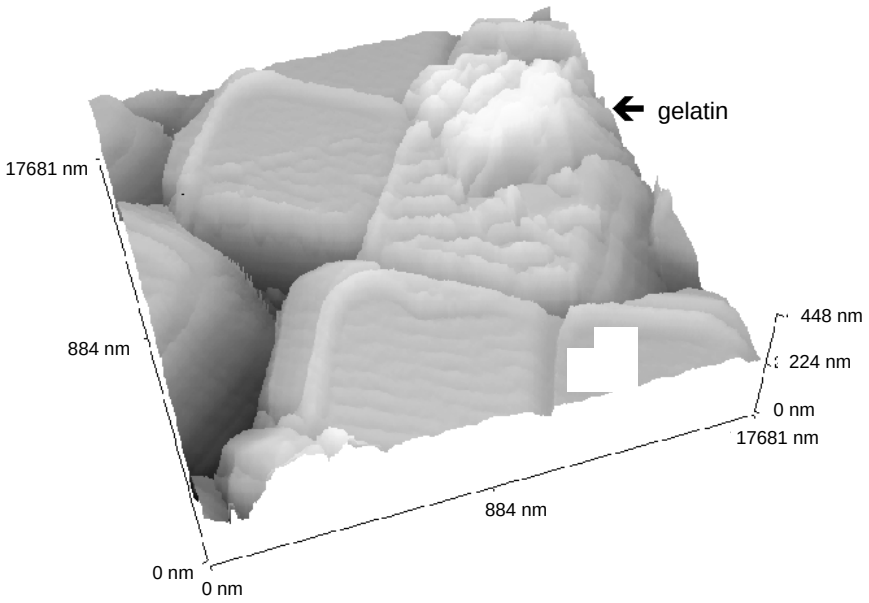

Figure 1. AFM image of gelatin mass remaining on the surface of AgBr. The low profile lines are thiacarbocyanine J-aggregates. See Ref. 9.

science. The specimen was received following several dilutions with warm water accompanied by vigorous agitation and elimination of dissolved gelatin by decantation. Finally, a two-dimensional AgBr particle layer formed floating on the water.[7] This thin particle layer was transferred onto an electroconductive ITO (Indium–Tin Oxide) plate. During the process, most of the gelatin adsorbed on the surface exposed to warm water is washed out and only the gelatin sandwiched between the particles remains.

The AgBr thin film was studied with a Hitachi S-5000 scanning electron microscope (HR-SEM) with 0.7 to 1.5 keV, 3 nm in diameter beam at room temperature.[8] A TopoMetrix TMX-2000 atomic force microscope (AFM) was also used in the contact mode.[9] The 670-nm laser light used in AFM does not expose photographically the AgBr specimen.

Results and Discussion

Though a substantial gelatin mass[4] is left in the specimen by enzyme treatment with centrifugal separation, the present specimen prepared by the washout method[7] contains few piles of gelatin fragments. An example of the rare massive gelatin remaining on the surface is shown in Fig. 1. Low-profile stripes aligned parallel on the surface are J-aggregates of thiacarbocyanine.[9] No ordered structure was found on the roof face of the gelatin mass.

As observed in the previous study,[4] a thin gelatin layer wrapping the AgBr particles is seen in a close-up image (Fig. 2). This thin gelatin layer prevents each particle from directly touching another. The warm water washing continues to dissolve the gelatin layer, and gelatin filaments begin to detach. With increasing distance between particles, gelatin films receive more solvent attack (here water), and the film edge becomes concave because of the surface tension of gelatin (arrows in Fig. 2). With progress of the wash out process, gelatin becomes sparse. Figure 3 shows how the continuous thin layer of gelatin (Fig. 2) becomes threaded. The area indicated by the wide arrow contains many strands to form netlike gelatin. The attack of the surrounding solvent water dissolved the

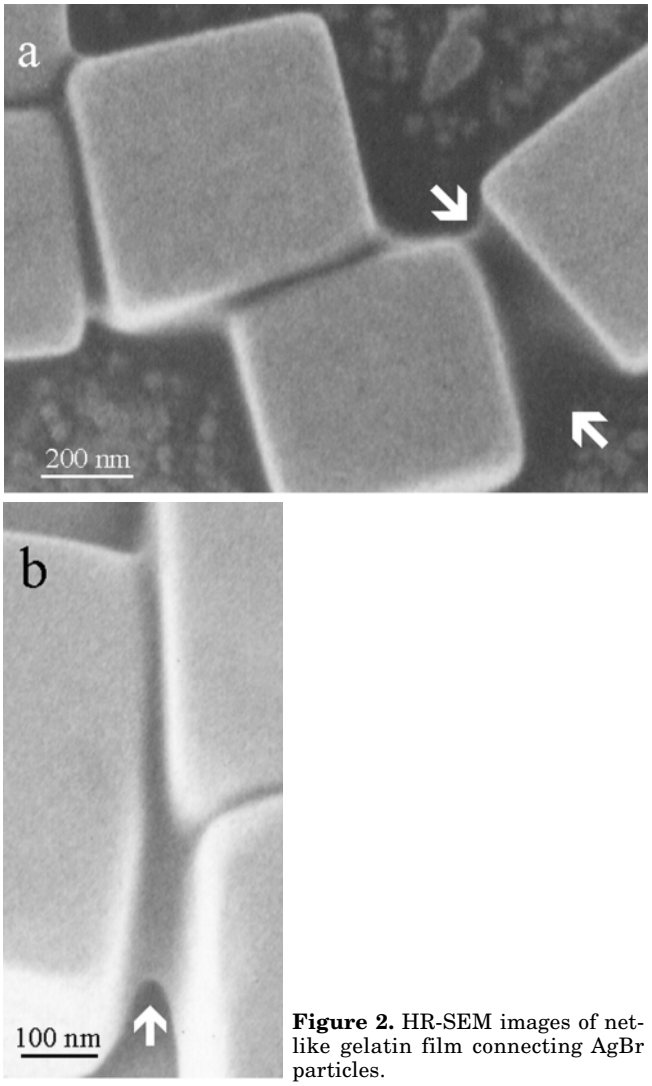

Figure 2. HR-SEM images of netlike gelatin film connecting AgBr particles.

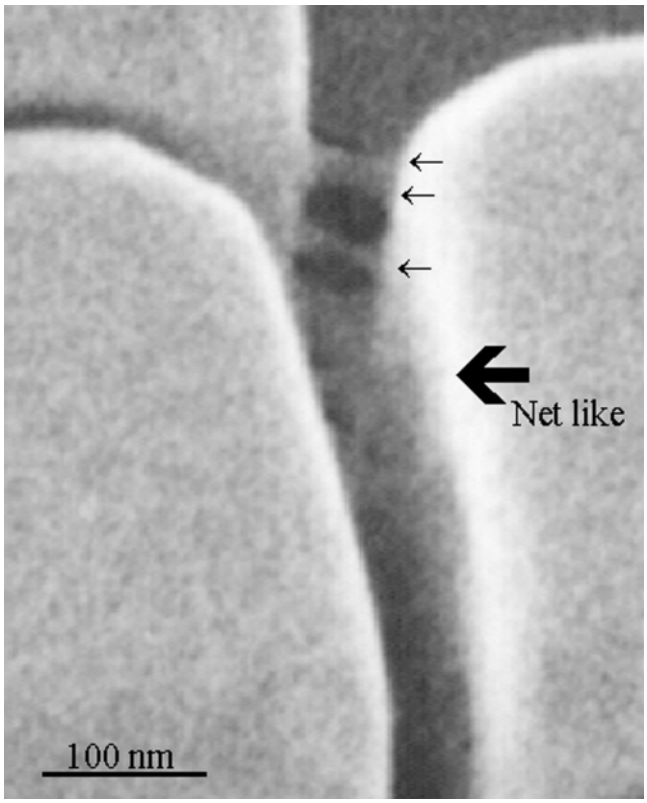

Figure 3. Most net-like gelatins are washed out by agitation in warm water and some strands remain bridging particles. The diameter at the thinnest point is about 20 nm.

gelatin filament and the gelatin remaining is in thin threads shown by the small arrows.

A typical image prepared by Heki's method[7] of a two-dimensional AgBr particle monolayer film is shown in Fig. 4. Close-packed particles seem to have a strong adhesive material between them to coalesce to each other. The adhesive material must be a gelatin thin layer as observed in Figs. 2 and 3. Thin strands of gelatin bridge loosely packed particles in the two-dimensional arrangement. The diameter of each strand is not uniform throughout their length, i.e., the strands have thick roots and narrow center portions. The narrowest diameter at the center in this figure is about 20 nm, which agrees well with the previous observation.[4]

AFM observations confirmed those of the SEM. Fibrous gelatins are clearly imaged bridging between or adsorbed on the side planes of AgBr particles in Fig. 5. The narrowest strand is 20 nm in diameter, and two or more strands run parallel to form wide bands (marked as "multi-strand" in the figure). The flat continuous one in the figure must be netlike gelatin as observed in Figs. 2 and 3.

Figure 6 shows enlarged SEM images of gelatin strands bridging AgBr particles. Clearly the shorter strand is thicker than the longer one and the adsorbing points (roots) are wider than the center. The diameter at the center of the longest strand is again 20 nm.

The gelatin strands remaining in the previous figures are adsorbed at or near the edges of the AgBr surfaces. It is not possible to observe gelatin between closely adhered particles. We also cannot expect to observe residual gelatin where surrounding water can easily attack to dissolve it away. Hence, we observed gelatin strands only where the particles were positioned slightly apart. The opposing

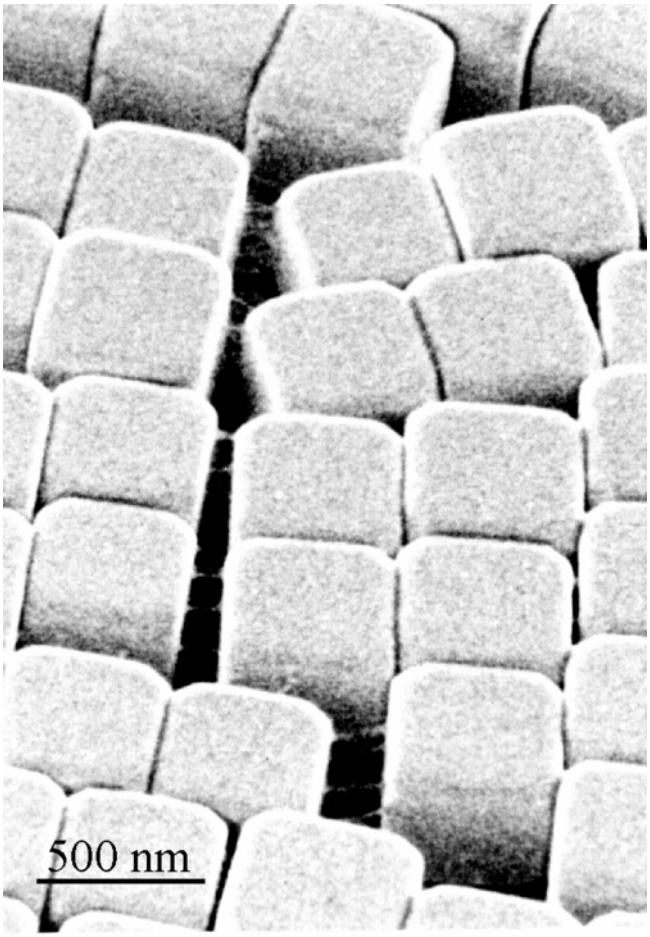

Figure 4. Two-dimensional arrangement of AgBr particles formed by the adhesion of gelatin. Unwashed fibrous gelatin are clearly seen between the loosely packed regions. On the top surfaces, the gelatin is washed out and no fibrous materials seem to remain.

faces of two particles are usually tilted toward each other and therefore one edge of a surface is closer than the other. The solvent water attacks from the wider side of the gap of the two facing planes (Fig. 2), and the gelatin strands remain only where the particles are positioned to form narrow gaps with their edges or corners. That is why we observe residual gelatin strands at the edges of AgBr particles in a two-dimensionally aligned film.

If a particle is positioned tilted from the two-dimensional particle plane of AgBr, residual gelatin strands are found in the center of the plane of AgBr (Fig. 7). A particle marked "A" positioned off plane and gelatin strands remain only where the two particles come close. At the root of strand on "A," a small mass of shrunk gelatin strand is seen. This shrunk strand was once bridged to another particle, but the attack of water caused breakage somewhere between the ends adsorbing to the surface. Another example of broken strands is in Fig. 8. A clump of shrunk fibrous gelatin adsorbs at the corner of the AgBr particles.

Gelatin is a highly structured assembly of collagen, and three collagen molecules make up a unit structure (Fig. 9). Because the diameter of one collagen triple-helix filament is about 2 nm, the 20-nm diameter of the narrowest fibrous gelatin observed between AgBr particles in this study must be a multiple thread of collagen helices. The present study cannot show details of the strands at the

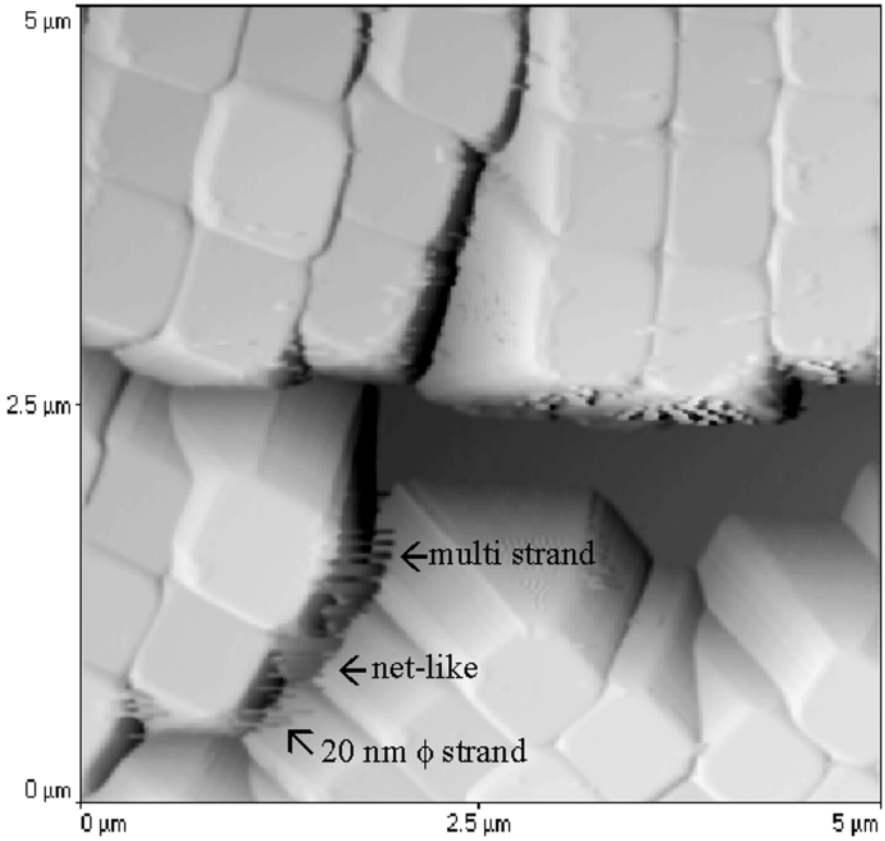

Figure 5. AFM image similar to Fig. 3. Net-like film and a multistrand of gelatin are shown. The thinnest strand has a diameter of 20 nm.

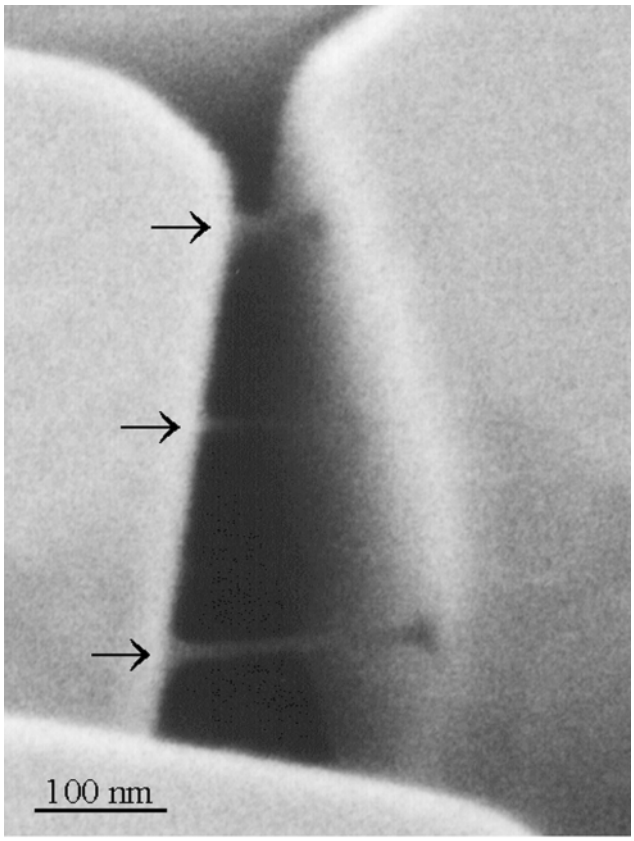

Figure 6. Enlarged images of bridging gelatin rod. The diameter for the longest one is about 20 nm.

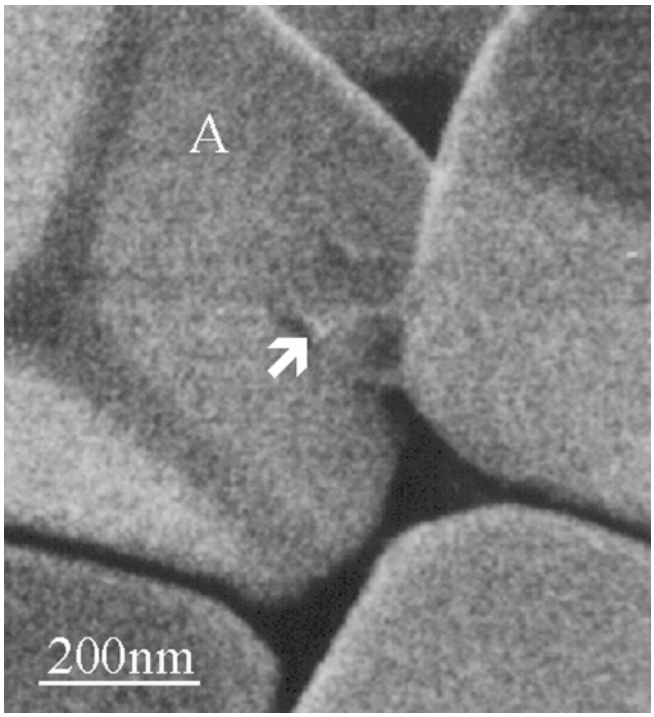

Figure 7. An example of gelatin adsorbed on the center of the particle marked "A." Gelatins adsorb anywhere on the surfaces but remain only where particles are close to each other. At the foot one strand marked by an arrow, a shrunk fragment of gelatin makes stem.

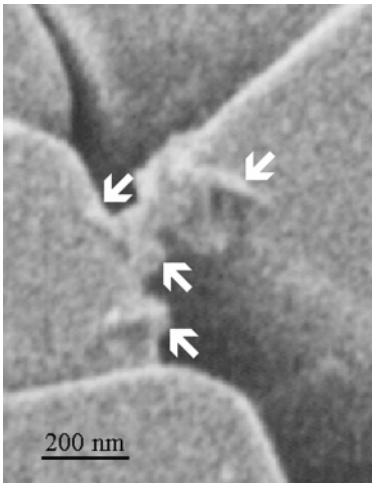

Figure 8. Shrunk fragment remain after break- age of a fibrous gelatin network.

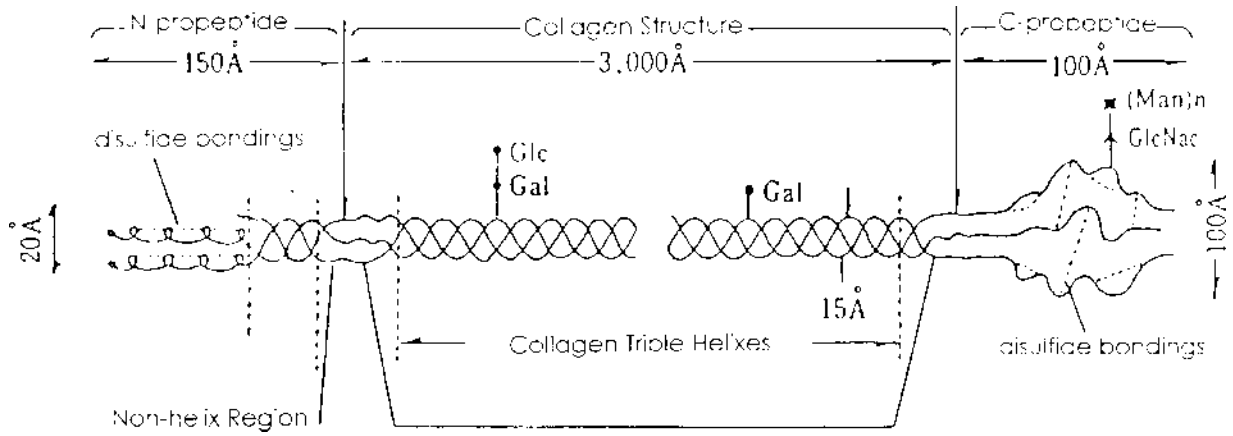

Figure 9. A schematic diagram of collagen triple helix, which is a basic unit of gelatin filament. This unit makes up the high-order structure of gelatin matrix. (Taken from the publication of Nitta Gelatin Corporation.)

resolution required to observe the collagen triple-helix filament. The strand might be like a tubular net woven with collagen helices where the two ends adsorb on AgBr surfaces. With stretching, the "gelatin net" becomes narrow at the center, and collagen helices in the fibrous gelatin will detach gradually from the surface of AgBr to dissolve away under agitation in water.

Conclusion

In warm water with strong agitation, the gelatin is removed as follows: The gelatin matrix is dissolved in water, and a thin gelatin layer remains to cover the AgBr particles to protect them from touching each other. Then, the thin cover layer is exposed to washing with water, and the network of gelatin strands in the thin layer begins to become fragmented. Most fragments are again washed out and ones that are about 20 nm in diameter, reside on the least washed sites.

The warm water washing process to remove gelatin provides better exposed surfaces of AgBr with little gelatin residue than the conventional method of enzyme decomposition followed by centrifugal separation.

Acknowledgment. Specimens were kindly prepared by Ms. S. Watanabe and Dr. T. Tani of Fuji Photo Film Co. Ltd. The HR-SEM observation with the Hitachi S-5000 was made by Ms. M. Nakagawa and Mr. M. Yamada at Hitachi Instruments Engineering Co. and Dr. M. Hasemi made the AFM observation with the TopoMetrix TMX-2000 at Nissei Sangyou Co. The authors appreciate their cooperation.

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