ELECTROCHEMICAL AGGREGATION OF TROPOCOLLAGEN.

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Summary

Tropocollagen aggregated and formed transparent membrane on the surface of the cathode by electrolyzing of the tropocollagen solution. Electron micrograph of the aggregate did not show an axial repeating period of about 700 A. observed in the native one. Furthermore, the wide-angle X-ray diagram of the aggregate gave diffuse rings. Therefore, the aggregate may be composed of a random arrangement of tropocollagen molecules.

It is well known that proper manipulation of such factors as pH[1], temperature[2], ionic strength[3] and addition of adenosine triphosphate (ATP)[4], induces the formation of various collagen aggregates from tropocollagen solution. Recently, it was found that the transparent aggregate was formed on the surface of the cathode by electrolyzing of the tropocollagen solution. The effect of factors on the electrochemical aggregation and the aggregate structure are presented in this paper.

MATERIAL AND APPARATUS

The preparation of the tropocollagen was based on the method of Kubota and Kimura[5]. The skin of the matured great blue shark, Prionace glauca, was scraped to remove all adhering fat, muscle and scale. The skin was then ground finely with a meat chopper. Globular proteins were extracted from the tissue with 100 volumes of 0.5 M sodium acetate solution for 24 hrs. The suspension was filtered through several layers of the cheesecloth and the filtrate

was discarded. The tissue was washed sufficiently with distilled water. Tropocollagen was extracted from the tissue with 10 volumes of 0.5 % acetic acid for 48 hrs.

The residue was removed from the extract by several layers of the cheesecloth and the filtrate was centrifuged at 55,000 x g for 2 hrs. The supernatant was dialyzed against 1000 volumes of 0.02 M disodium hydrogen phosphate for three days. The collagen fiber precipitated during the dialysis was collected by centrifugation at 3,000 x g for 20 mins. The collagen fiber obtained was washed with distilled water thoroughly. This collagen fiber dissolved in 0.0001 N hydrochloric acid solution and finally the pH of the solution was ajusted to pH 3.5 with 0.01 N hydrochloric acid solution. All experiments were performed at $5^{\circ}C$.
The collagen content or the amount of the aggregate was calculated from the nitrogen content of the solution or the aggregate by the micro-Kjeldahl method.

A cylindrical glass cell (ϕ 4cm X 5.5cm) was used for experiments. The cell contained two smooth platinum electrodes (4cm X 2cm), held 3.5 cm apart to produce an approximately uniform field[6].

In most of the experiments described here, direct constant current of 32 mA was used. Constant current was supplied with current stabilizer (Toyo Roshi Co.). 50 ml of 0.1 % tropocollagen solution was employed in each experiments. The solution was not stirred during electrolysis. The electrolyte was cooled in ice to avoid the denaturation of the collagen.

X-ray diffraction diagrams were obtained with Rigaku Denki X-ray generator unit. Wet aggregates formed electrochemically were dried up in a vacuum and exposed for 1 hr. to the X-ray beam with a sample-to-film distance of 10 cm.

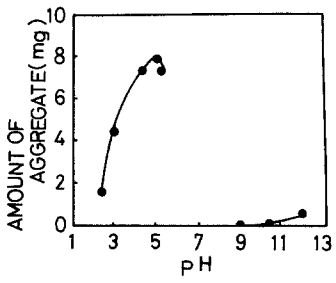

Fig. 1.

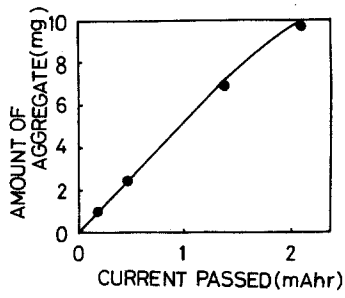

Fig. 2.

Figure 1. Effect of pH on collagen aggregation. 50 ml of 0.1 % tropocollagen solution was employed, current of 32 mA passing throug cell for 2 mins.

Figure 2. Relationship between current passed and amount of aggregate. 50 ml of 0.1 % tropocollagen solution (pH 3.3) was employed, current of 32 mA passing through cell.

Electron micrographs were obtained with Hitachi HSM-2 electron microscope at 50 kv of accelerating voltage. Samples were stained with 0.2 per cent phosphotungstic acid (PTA).

RESULTS AND DISCUSSION

Collagen, under a high-potential direct current, migrated toward an electrode and aggregated on the surface of the electrode. The aggregate was a transparent membrane.

Figure 1 shows the effect of pH on collagen aggregation. As is evident, collagen aggregated on the cathode from a pH of 2.5 to 5.3, and on the anode from a pH of 9 to 12. The amount of the aggregate was maximum at pH value of about 5 in the acidic condition. However, in the alkaline condition, the aggregate would slide off the electrode as soon as the electrode was lifted from the cell. Therefore, the amount of the aggregate could not be determined.

Figure 2 shows the relationship between the current passed and the amount of the aggregate. The amount of the aggregate

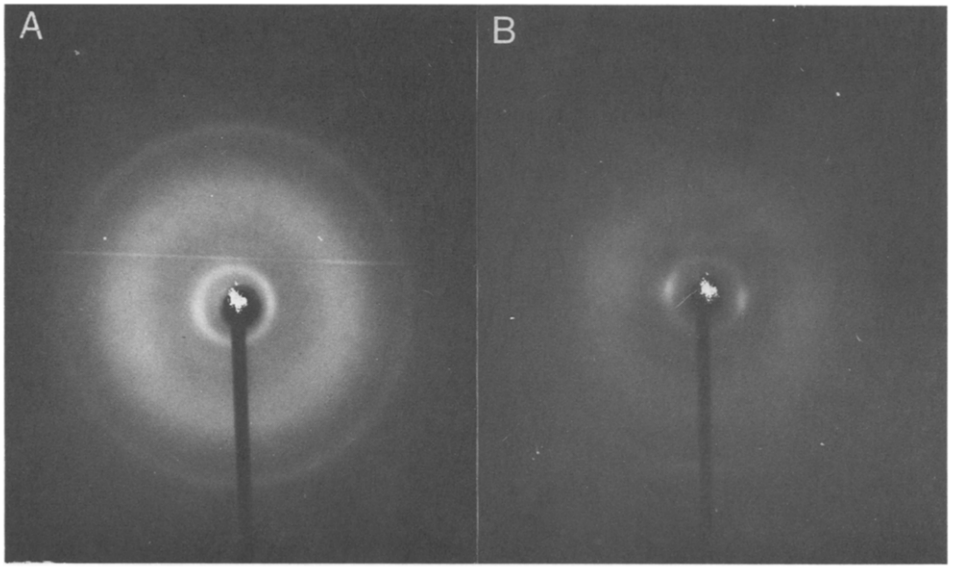

Figure 3. Wide-angle X-ray diagram of aggregate formed electrochemically.
A: allowed to shrink freely.
B: stretched and dried under tention.

increased linealy with the increase in the current passed. When the current passed increased, almost all tropocollagen in the solution aggregated on the surface of the cathode. However, the aggregate was not formed electrochemically in the presence of more than 1.5 m moles per liter of salts, such as sodium chloride, potassium chloride and calcium chloride, because it prevented the electroendosmotic dehydration from the aggregate[7].

The wide-angle X-ray diagram of the chemically formed aggregate prepared from shark tropocollagen solution by dialyzing against 0.02 M disodium hydrogen phosphate solution for 48 hrs was very similar to those of other collagen[8]. The wide-angle X-ray diagram of the aggregate formed electrochemically (sample A in Figure 3) showed rings, indicating random orientation of macromolecules followed by a diffuse scattering of the diffracted

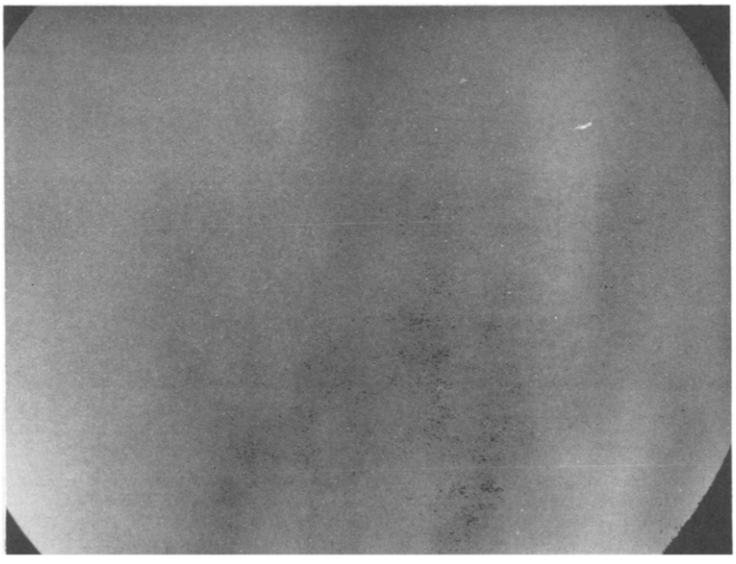

Figure 4. Electron micrograph of aggregate formed electrochemically. (x 57,000)

X-ray[9], of approximately 2.86 A., 4.5 A. and 11 A. However, the wide-angle diffraction pattern of the aggregate which had been stretched and dried under tension was similar to that of the native fiber (sample B in Figure 3). The appearance of the 2.86 A. arc on the meridian, the 11 A. equatorial spot and the diffuse "halo" of about 4.5 A. in the sample B means that, as a result of stretching, macromolecules have lined up along the axis of stretching. Furthermore, small-angle diffraction photographs of the aggregate indicating crystalline arrangement could not obtained.

The structure of the aggregate was also studied by the electron microscope. The aggregate formed chemically consisted of fine fibrils showing an axial repeating period of about 700 A. However, as shown in Figure 4, a visible repeating period which seemed to be caused by regular arrangement of tropocollagen

molecules[10] was not observed on electron micrographs of the aggregate formed electrochemically.

Those results, described above, suggested that tropocollagen molecules might be arranged at random in the aggregate.

The aggregate formed electrochemically could be redisolved in hydrochloric acid solution (below pH 4). The redisolved collagen in dilute hydrochloric acid solution (pH 3.5) was tested for their ability to form native type (700 A repeat) fibrils by dialyzing against 0.02 M disodium hydrogen phosphate solution for 48 hrs. at 5°C. Precipitates were formed from the redisolved collagen solution by dialysis. The resulting precipitates were stained with 0.2 per cent PTA and examined in the electron microscope. Electron micrograph of the precipitate was composed of fibrils of an axial repeating period of about 700 A.

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