Studies on the Silk-Palladium Catalyst. III. Physicochemical Characterization

By Yoshiharu IZUMI

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In the preceding papers of this series[1,2], it was reported that silk and 6-Nylon-palladium catalysts are in a number of respects superior as hydrogenation catalysts to carbon-palladium and other palladium catalysts hitherto described. It was also suggested that these catalysts resemble enzymes in that their activity is considerably affected by metal ions and other agents and some of them are endowed with an ability to catalyze asymmetric hydrogenation reactions[3-5].

It, therefore, seemed of special interest to study their structure and the process of their activation from physicochemical points of view. It was also hoped that such studies would shed some light on the mechanism by which some of these catalysts achieve the asymmetric reactions.

The present paper describes X-ray diffraction, infrared spectrophotometric experiments on silk fibroin,its palladous chloride chelate compound, and silk-palladium catalyst (samples of silk series) and on the corresponding samples of 6-Nylon* (samples of Nylon series). The behaviors of these samples towards *p*-nitrosodiethylaniline (PNDA), a specific reagent for palladium[6], were also included in this paper.

Results and Discussion

X-Ray Diffraction Studies. — X-ray diffraction pictures of fibrous preparations of the silk series and the Nylon series are shown in Figs. 1 and 2, respectively. X-ray diffraction measurements were also made on powdered samples of the silk series using a Norelco X-ray instrument and the charts obtained are reproduced in Fig. 3.

1) Y. Izumi, This Bulletin, **32**, 932 (1959).
2) Y. Izumi, ibid., **32**, 936 (1959).
3) S. Akabori, S. Sakurai, Y. Izumi and Y. Fujii, *Nature*, **178**, 323 (1956).
4) S. Akabori, Y. Izumi, Y. Fujii and S. Sakurai, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **77**, 1374 (1956).
5) S. Akabori, Y. Izumi and Y. Fujii, ibid., **78**, 886 (1957).
* Polycaprolactum.
6) L. G. Overholser and J. H. Yoe, *J. Am. Chem. Soc.*, **63**, 3224 (1941).

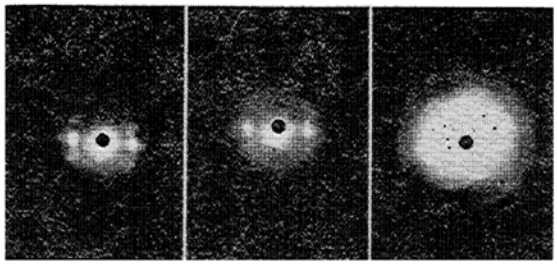

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Fig. 1. X-ray diffraction pictures of silk series.

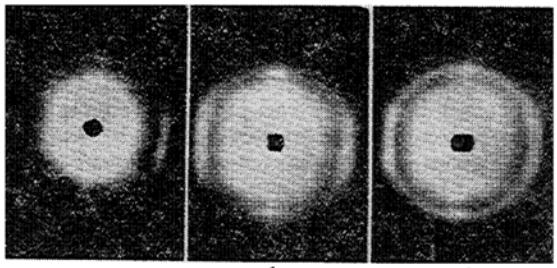

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Fig. 2. X-ray diffraction pictures of Nylon series.

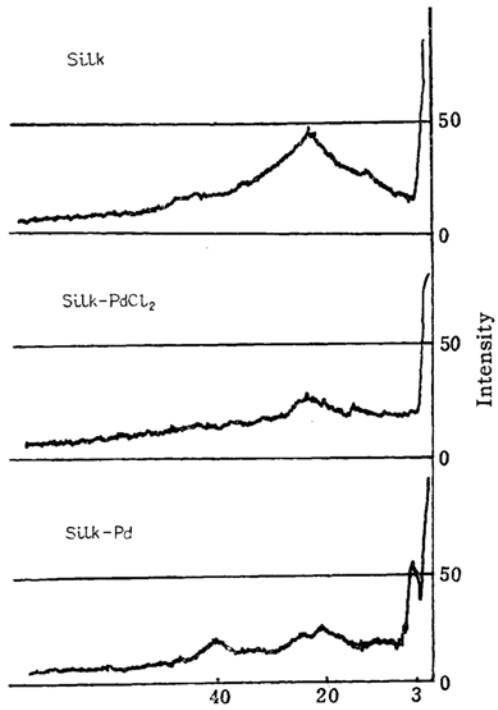

Fig. 3. X-ray diffraction patterns of powdered silk series.

As has been known for some time, Figs. 1a and 2a clearly indicate that both silk fibroin and 6-Nylon fibers contain crystalline parts in their structures. The X-ray fiber diagrams of palladous chloride chelates of silk fibroin and 6-Nylon are essentially the same with those of silk fibroin and 6-Nylon, respectively, suggesting that the chelate formation with palladous chloride does not significantly alter the arrangement of these fibers. Conversion of these chelate compounds to catalytic active forms by hydrogenation, however, seems to cause some changes as can be judged from the distinct patterns due to palladium atoms seen in the diagrams of the catalysts.

The Norelco patterns of powdered samples of the silk series are in good agreement with the fiber diagrams of the corresponding preparations. It is noted that the pattern for the powdered silk-palladium catalyst shows in the low-angle region (ca. 3.5°), a peak of which may be due to a ca. 25 Å regular arrangement of the structure.

In summary, it may be concluded that a part of the palladium atoms in the silk-palladium catalysts are assembled in fine crystalline particles and the other part of the palladium probably becomes regularly arranged in the micelle structures of the fibers. However, the possibility that certain regular structures have emerged in the silk protein itself can not be excluded.

Infrared Absorption Measurements. — For the infrared absorption studies powdered samples of the silk series and thin sheet forms of the Nylon series were employed. The spectra of these samples are shown in Fig. 4. As a reference substance carbobenzoxy triglycylleucine and its palladous chloride complex were chosen, the spectra of which are shown in Fig. 4.

Generally speaking, the infrared absorption spectra of the samples of the silk series did not show any remarkable differences, probably due to the high polymeric nature of silk fibroin. Especially, the spectra of the Nylon samples were quite similar to each other, because of the fact that the measurements were made on thin sheet samples, and they contained only a small amount of palladium. However, it can be pointed out, on close examination of the spectra, that the powdered silk-palladium catalyst is similar to the silk fibroin powder rather than to the chelate preparation. A comparison of the infrared absorption spectra of carbobenzoxy triglycylleucine (Cbz. Trigly-Leu) and its palladous chloride complex on one hand and silk fibroin and its palladous chelate on the other hand reveals that an absorption at 3000 cm^{-1} region, which is due

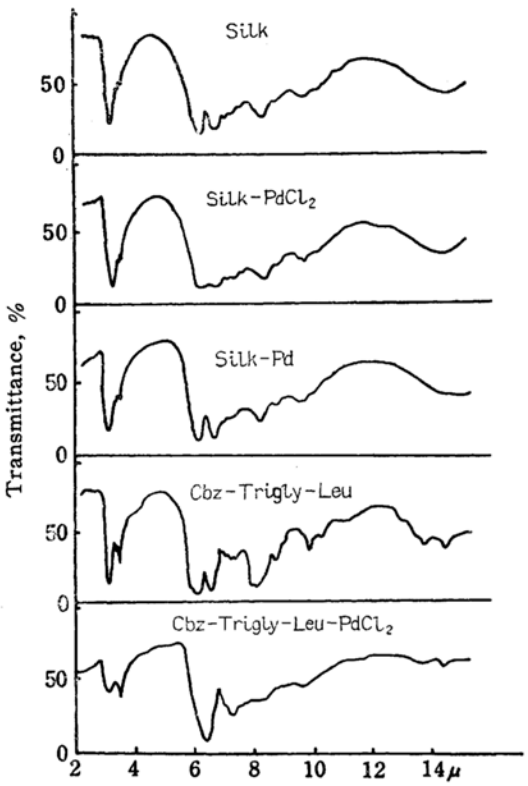

Fig. 4. IR-spectra of silk, silk-$PdCl_2$, silk-Pd, Cbz-Trigly-Leu and Cbz-Grigly-Leu-$PdCl_2$.

to NH groups (probably also OH groups) decreases on the formation of chelates. This suggests that the NH groups (probably also OH groups) of both silk fibroin and the carbobenzoxy tetrapeptide are involved in the chelation with palladous chloride. It may also be noticed that in the spectra of chelate compounds, absorptions at 1640 and 1530 cm^{-1} are diminished and that at 1590 cm^{-1} is increased as compared with the spectra of unchelated forms. The absorptions at 1640 and 1530 cm^{-1} are usually interpreted as caused by CO in COOH and CONH groups, and the absorption at 1590 cm^{-1} as caused by COO^- groups, and Gierer[7] has reported that the complex formation of an amide results in a shift of the absorptions due to CONH to longer wave-lengths. It is therefore probable that the observed changes in the region of 1530 to 1640 cm^{-1} are due to a complicated effect of chelate bond formation between palladous chloride and COOH and/or CONH groups.

It may be concluded that palladous chloride forms chelate linkages with NH_2 (-OH), CONH and COOH groups of fibroin and the dipeptide. These linkages appear to be broken on hydrogenation of the chelate. In the activated chelate, therefore, the palladium seems to be in an almost atomic state.

Behaviors towards *p*-Nitrosodiethylaniline (pNDA). — When silk fibroin and Nylon-$PdCl_2$ chelate compounds were treated with pNDA, a specific reagent for palladium, parts of palladous chloride were released. However, the rest of palladous chloride remained in fibers, although the fibers were strongly colored like pNDA-$PdCl_2$ complex. Furthermore, Fujii[8] observed that silk fibroin-$PdCl_2$ chelate treated with pNDA is more active than silk fibroin-$PdCl_2$ itself and pNDA in catalyzing the decarboxylation of oxalacetic acid. These facts suggest that a considerable part of the palladium is firmly kept in the fiber structure and that on treatment with pNDA the reagent becomes combined with silk fibroin through the mediation of palladium atom or pNDA-$PdCl_2$ complex becomes wrapped into the fiber micelles.

In summary, it can be inferred that palladous chloride is first dispersed in the fiber structures because of the chelate formation. On reduction, palladous chloride in the chelate is converted into an atomic state leaving the carrier protein.

Experimental

I. Preparation of Powdered Silk Fibroin Preparations. — One gram of silk fibroin was dissolved in a modified Schweizer reagent made from 1.6 g. of ethylenediamine and 1.2 g. of $Cu(OH)_2$, and the solution was neutralized with acetic acid and dialyzed. Five milliliters of 1% ethylene diamine tetraacetate solution and 0.1 g. of celite were added to the dialyzed solution and the mixture was filtered under suction. To the filtrate were gradually added 3 volumes of acetone and the precipitate formed was collected by centrifugation. The precipitate was dried at 50°C after washing 4 times with acetone and then once with ethyl ether. The dried silk fibroin was ground in a mortar and passed through a 200 mesh sieve. 0.5 g. of finely powdered silk fibroin was obtained.

This powdered fibroin was converted to palladous chloride chelate and catalyst forms according to the standard methods described in Part I. The chelate was brown in color, and the catalyst resembled active carbon in its appearance.

II. Preparation of 6-Nylon Catalyst Samples for X-ray Measurements. — In order to prevent the tangling of the chelate fibers during the reduction, they were fixed to a holder of the X-ray apparatus. After measurement of the

7) A. Gierer, *Z. Naturforsch*, 8b, 654 (1953).

8) Y. Fujii, *J. Biochem.*, in preparation.

X-ray diffraction, the holder with the chelate fibers in it was wrapped in gauze and then reduced in the autoclave.

III. 6-Nylon-$PdCl_2$ Complex.—The complex was prepared analogously to the silk fibroin-$PdCl_2$ complex.

IV. Triglycyl-carbobenzoxyleucine-$PdCl_2$ Complex. — Forty-four milligrams of triglycyl-carbobenzoxyleucine was boiled for 4 min. with 140 ml. 0.1 N acetic acid containing 140 mg. palladous chloride and the resulting peptide-$PdCl_2$ complex was isolated centrifugally and washed completely with water and then with methanol and dried.

V. X-ray Diffraction Measurements. — X-ray fiber photographs were taken with K_α line of copper filtered through a nickel filter. For powdered samples a Norelco X-ray instrument was used.

VI. Infrared Absorption Measurements. —A Perkin-Elmer apparatus was used. The measurements were made by the KBr tablet method.

Summary

1. From the infrared absorption measurements of silk fibroin, silk fibroin-$PdCl_2$ complex, and silk-palladium catalyst, it was concluded that the NH, OH, COOH and CONH groups of the fibroin participate in the chelate formation with palladous chloride. On reduction of the chelate with hydrogen, the chelate bonds are broken and the fibroin restores its original form.

2. X-ray diffraction pictures of the above-mentioned samples and of the corresponding preparations of the 6-Nylon series suggest that a part of the palladium atoms in the silk-palladium catalyst is assembled in fine crystaline particles and the other part of the palladium probably becomes regularly arranged in the micelle structures of the fibers. However, the possibility that certain regular structures have emerged in the carrier protein itself can not be excluded.

3. Part of the palladium in silk fibroin-$PdCl_2$ complex can be readily released with *p*-nitrosodimethylaniline, while the rest appears to be strongly kept in the fiber structure.

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Division of Organic Chemistry
Institute for Protein Research
Osaka University
Nishi-ku, Osaka