The Ultrastructure of Amyloid Substances from Bovine Kidneys

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ABSTRACT. Amyloid substances were extracted from bovine amyloid-laden kidneys and examined for their morphological characteristics. The substances were shown to contain amyloid fibrils with subunits of protofibril structure, about 2 to 2.3 nm wide, which were non-branching and almost uniform in width, approximately 13 nm(8 to 18 nm), having no periodicity. In addition, amyloid p-component was also demonstrated in doughnuts- or ring-like structure, about 10 nm in the outside diameter and 5 nm in the inside diameter.—*Key words:* amyloid substance, ultrastructure.

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INTRODUCTION

Amyloid substances (AS) have been demonstrated to be composed of amyloid fibrils (AF) with the subunit of protofibril structure [18]. In addition to AF, human [4, 7, 8, 19] and murine [14] AS have been recognized to contain amyloid p-components (AP, so-called plasma component) which are pentagonal shaped units measuring 9 nm in the outside diameter and 4 nm in the inside diameter. However, there have been no descriptions concerning protofibrils and AP in bovine AS. The present communication describes the electron microscopic observations on negatively stained AS from bovine amyloid-laden kidneys (AK).

MATERIALS AND METHODS

Bovine kidneys with severe amyloid deposits in the glomeruli and moderate in the medullary regions were used for the isolation of AS according to the methods of Takahashi *et al.* [20] and Skinner *et al.* [19]. The procedure of AS extraction is summarized in Fig. 1. AK were sliced and homogenized in 0.15 M NaCl solution in ultradisperser (Model LK-1, Yamato Co., Japan) with external cooling at 4°C. The homogenate was centri-

Fig. 1. Procedure for isolation of amyloid substances.

fuged at 12,000 g for 20 minutes and the supernatant solution was discarded. The precipitate was homogenized and centrifuged repeatedly as described above until the result-

Fig. 2. Amyloid fibrils in separate. Negative stain. $\times 30,000$.

Fig. 3. Amyloid fibrils in pairs. Negative stain. $\times 30,000$.

ing supernatant solution became transparent, with its absorbance of less than 0.1 at 280 nm. The top layer of the last sediment was separated to homogenize in 1.5 M NaCl solution and stirred for 12 hours to deprive cell components. Thereafter, the mixture was centrifuged at 38,000 g for 20 minutes. Homogenization and centrifugation was repeated again until the supernatant became transparent with its absorbance of less than 0.05 at 280 nm. The last sediment was transferred into 0.15 M NaCl solution, and homogenized and centrifuged twice or 3 times. This solution was added with collagenase (1 mg/50 to 100 mg of protein, extracted from *Clostridium histolyticum*, grade II, Boehringer Mannheim, W. Germany) and also supplemented with CaCl_2 at a final concentration of 1 mM, and then stirred for 1 hour at room temperature and for 12 hours at 4°C. Homogenization and centrifugation was repeated 3 times in the same manner as described above. The residue was then homogenized with distilled water and centrifuged at 40,000 g for 10 minutes, and treated in the same way 5 times. These supernatants were pooled to dialyze against distilled water and lyophilized as specimen I. The supernatant solutions from homogenate of sliced AK in 0.15 M NaCl solution in this procedure were pooled and lyophilized for the specimen II.

The lyophilized specimen I was smeared and stained with alkaline Congo red [15], and observed under polarized light microscopy. The lyophilized specimens I and II were stained negatively with 2% phosphotungstic acid (pH 5.2) [18] and examined in JEOL-100S electron microscope at 80 kv.

RESULTS

Light microscopic observations on alkaline Congo red stained samples from specimen I revealed green birefringence. Electron microscopic observations on the negatively stained samples from specimen I showed lots of fibrillar substances. They were non-branching and almost uniform in width, approximately 13 nm (8 to 18 nm), having no periodicity unlike collagen fibers. The fibrils were commonly in separate (Fig. 2) and sometimes in double strands (Fig. 3), showing ribbon-like appearance with periodic twisting each other in about 280 nm (240 to 320 nm) interval (Fig. 4). High magnification of this fibril ($\times 200,000$) revealed fibrillar structure as the subunit, about 2 to 2.3 nm in width (Inset in Fig. 4).

Investigation of the samples from the specimen II showed a lot of doughnuts-like structures with approximately 10 nm in outside diameter and 5 nm in inside diameter (Fig. 5).

Fig. 4. Amyloid fibrils with twists at 240 to 320 nm intervals, showing ribbon-like appearance. Negative stain. $\times 40,000$. Inset: Subunit structures of protofibril. Negative stain. $\times 200,000$.

Fig. 5. Doughnuts- or ring-like structures of amyloid p-component. Negative stain. $\times 150,000$.

DISCUSSION

Negatively stained samples from the top layer of the sediment revealed a lot of fibrillar substances. These fibrils were considered to be AF since they showed green birefringence in alkaline Congo red staining [15] and the morphological findings including their width were similar to those deposited in tissues [9, 11, 13].

The twisting of the double strands of fibrils was also detected as in bovine [10], murine [20], and human [18] AF. The range of the width in bovine AF in the present study were 8 to 18 nm and slightly wider than those reported to be 5 to 10 nm in human [4, 5, 17, 18], 7.5 to 8.5 nm in experimental murine [20] and 8 to 10 nm in canine [12] AF, respectively.

The subunit structure with approximately 2 to 2.3 nm width shown in bovine AF was considered to be protofibril structure as described in human AF by Shirahama *et al.* [18].

Lots of doughnuts-like or ring-like structures in the specimen II from the supernatant of homogenate with 0.15 M NaCl solution were regarded to be AP in the view point of morphologic characteristics as in the case of

human and murine AP [4, 7, 8, 14, 19]. The chemical analysis of bovine AP is recommended.

AP has been recognized in normal tissues [16] as well as in amyloid-laden tissues [6], and is known to be a certain constituent of human serum amyloid p-component [1–3]. However, the role of AP in the pathogenesis of amyloidosis still remains unknown.

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要 約

牛腎臓由来のアミロイド物質の微細構造: 金 徳煥・小野憲一郎・長谷川篤彦・友田 勇 (東京大学農学部家畜内科学教室)——牛の腎臓からアミロイド物質を抽出し, その形状を検索した。アミロイド物質には幅約 2~2.3 nm の原線維からなるサブユニット構造を有するアミロイド線維が見出された。本線維の幅は 13 nm (8~18 nm) で, 未分枝で周期性はなかった。また, アミロイド線維のほかに, 外径 10 nm, 内径 5 nm のドーナツ状あるいは環状のアミロイド P-成分も見出された。