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Isolation of Different Hydroxyproline Containing Proteins from Bovine Vitreous Body Collagen

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Abstract. From pepsin-solubilized vitreous body collagen three different precipitates were collected by differential salt precipitation. These three different protein fractions contain hydroxyproline and show different patterns in polyacrylamide gel electrophoresis suggesting different collagen types.

Zusammenfassung. Aus pepsin-gelöstem Glaskörperkollagen konnten mit Präzipitation aus Salzlösungen verschiedener Molarität drei verschiedene Präzipitate erhalten werden. Diese drei Fraktionen enthalten Hydroxyprolin und zeigen in der Polyacrylamidgel Elektrophorese verschiedene Bandenspektren. Daher wird vermutet, daß es sich bei den drei Präzipitaten um verschiedene Kollagentypen handelt.

Introduction

It has been suggested that of the five genetically distinct collagen types which have been isolated until now, collagen type II constitutes the residual protein of the vitreous body (Swann et al., 1972; Smith et al., 1976; Von der Mark et al., 1977). However, polyacrylamide gel electrophoresis of pepsin-solubilized fibers of the vitreous body and comparison of the cyanogen bromide peptides revealed no identity between vitreous body collagen and type II collagen (Hofmann and Schmut, 1977). It was concluded that the fibers of the vitreous body consist of a mixture of different collagen types. This was confirmed by the studies of Linsenmayer and Little (1978), who showed that chicken neural retina cells, assumed to produce vitreous body components, synthesize type II collagen and a new genetic type of collagen. The purpose of this investigation was to separate different collagen types of pepsin-solubilized vitreous body collagen by differential salt precipitation.

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Material and Methods

Bovine Vitreous Body Collagen

The residual protein of bovine vitreous body was isolated and extracted twice with 0.5 M sodium acetate and 0.5 M citrate buffer (pH 3.7) as described by Hofmann and Schmut (1977).

Solubilization with Pepsin and Differential Salt Precipitation

After extraction with sodium acetate and citrate buffer the vitreous body collagen residue was solubilized with 5 mg/g wet weight pepsin (2,500 U/mg, Boehringer/Mannheim Nr. 108057) in 0.5 M acetic acid at 4° C for 24 h. After centrifugation, a first fraction of the pepsin-solubilized collagen was precipitated from the supernatant by addition of solid NaCl to a final concentration of 0.7 M. After 24 h at 4° C, the residue was collected by centrifugation (3,000 g) and the NaCl concentration of the supernatant adjusted to 0.9 M. The precipitate was spun down (3,000 g), and a third precipitate was obtained from the supernatant at a NaCl concentration of 1.2 M. After centrifugation of the 1.2 M precipitate, a 2.0 M NaCl fraction was collected from the supernatant by addition of solid NaCl.

The 0.7 M, 0.9 M, 1.2 M, and the 2.0 M preicipitates were solubilized in 0.5 M acetic acid and reprecipitated with the adequate NaCl concentrations. The precipitates were collected by centrifugation, solubilized with H_2O, precipitated with acetone, washed several times with fresh acetone, and dried in air.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed according to Furthmayr and Timpl (1971) with 5.75% gels at 6 mA per tube for 2 h.

Hydroxyproline

Hydroxyproline was determined by the method of Stegemann (1958) after hydrolyzing the samples under nitrogen in 6 M HCl at 105° C for 16 h.

Results and Discussion

Differential salt precipitation of pepsin-solubilized vitreous body collagen in 0.5 M acetic acid permits the isolation of different protein fractions. We isolated and analyzed precipitates which can be obtained at NaCl concentrations of 0.7 M, 0.9 M, 1.2 M, and 2.0 M.

By using the method of Stegemann (1958), we found that all fractions contain hydroxyproline, indicating the collagenous nature of the separated proteins.

The different fractions were also investigated by polyacrylamide gel electrophoresis (Fig. 1). The 0.7 M precipitate shows in polyacrylamide gel electrophoresis only one α-band. The 0.9 M precipitate represents a mixture of the 0.7 M and the 1.2 M fraction and was not further investigated.

At a NaCl concentration of 1.2 M, a precipitate is formed which shows in polyacrylamide gel electrophoresis two chains in the α-position, designated as 3 and α in Fig. 1.

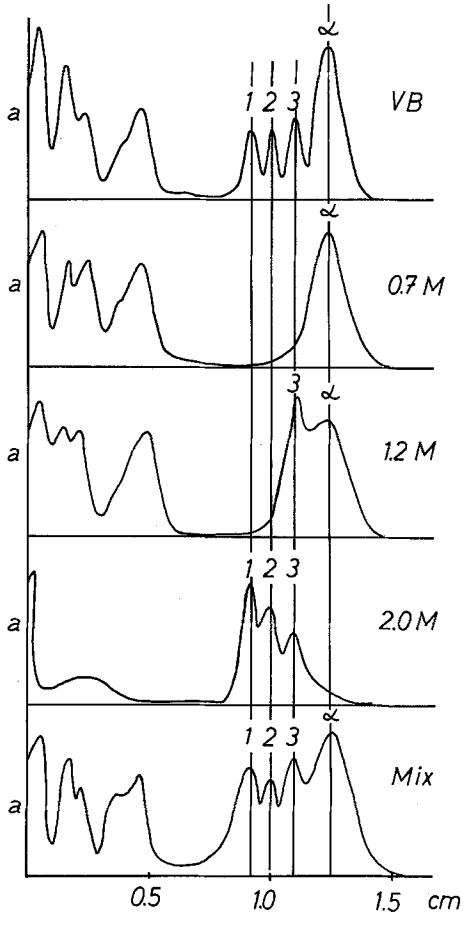

Fig. 1. Polyacrylamide gel electrophoresis of pepsin-solubilized vitreous body collagen (VB) and of the proteins obtained at 0.7 M, 1.2 M, and 2.0 M NaCl concentrations. A mixture of the 0.7 M, 1.2 M, and 2.0 M precipitates (Mix) shows the pattern of the native pepsin-solubilized vitreous body collagen

The 2.0 M precipitate consists of three bands migrating between the α- and the β-components and were designated as 1, 2, and 3 in Fig. 1.

Recombination of the 0.7 M, 1.2 M, and 2.0 M fractions shows the polyacrylamide gel electrophoresis pattern of native pepsin-solubilized vitreous body collagen (Fig. 1).

The migration distance of the α-component of the 0.7 M NaCl fraction corresponds with that of the α_1 (II)-chains. The occurrence of type II collagen in vitreous body fibers has been suggested from amino acid analysis of the vitreous body collagen by Swann et al. (1972). Also immunofluorescent studies (Von der Mark et al., 1977) and the CNBr-derived peptides of collagen produced by chick neural retina tissue (Smith et al., 1976) indicated the presence of type II collagen in vitreous body fibrils.

The migration distance of the peptide chains obtained by 1.2 M NaCl precipitation is closely related to the migration distance of the αA and αB chains of the collagen described by Burgeson et al. (1976), recently designated as type V collagen.

The 2.0 M NaCl precipitate shows a similar polyacrylamide gel electrophoresis pattern as the new genetic collagen type investigated by Linsenmayer and Little (1978).

From the present data one cannot establish whether the two protein chains of the 1.2 M fraction or the three chains of the 2.0 M fraction occur in a triple helical molecule like the five common collagen types.

However, the separation method presented in this paper allows the isolation of large quantities of fractionated material, and we suppose that this method will bring further information on the collagenous nature of the fibers of bovine vitreous body.

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