Hoppe-Seyler's Z. Physiol. Chem.
Bd. 357, S. 601 - 604, April 1976

SHORT COMMUNICATION

# Isolation of a Procollagen Peptide from Amniotic Fluid

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(Received 12 February 1976)

*Isolierung eines Prokollagen-Peptids aus Amnionflüssigkeit*

**Zusammenfassung:** Aus Rinderamnionflüssigkeit wurde ein Peptid mit einem Molekulargewicht von ca. 15000 isoliert. Dieses Peptid war immunologisch identisch mit dem aminoterminalen Peptid der pro α1 (I)-Kette aus Prokollagen und bestand aus einer globulären und kollagen-ähnlichen Region. Die Ergebnisse weisen darauf hin, daß der aminoterminale Teil des Prokollagens durch Proteasen als gesamte Einheit abgespalten wird.

*Key words:* Procollagen catabolism, peptide structure, radioimmune assay

Collagens are formed from larger biosynthetic precursors, the procollagens, which have additional peptide segments at the amino and carboxyl ends of each chain. Current evidence suggests that the precursor-specific peptides are removed en bloc[1-5] and persist for some time in the body[6,7]. Using immunological methods, materials reacting with antibody against determinants in the amino-terminal portion of the pro α1 (I) chain have been detected in biological fluids[8]. In the present study we have isolated the antigenically active materials from amniotic fluid and compared their structure with that of the amino-terminal peptide derived by cyanogen bromide cleavage of the pro α1 (I) chain[9].

## Materials and Methods

Bovine amniotic fluid was obtained from within the placental membranes of freshly slaughtered pregnant cows. Solid ammonium sulfate was added to 10 *l* batches and the proteins precipitating between 30 and 60% saturation were collected by centrifugation (30 min, 15000 × *g*). The precipitate was suspended in about 1 *l* of 0.1M acetic acid and dialyzed overnight against the same solvent. After centrifugation the supernatant fluid was dialyzed against 0.1M Tris/HCl, pH 7.4, 0.04M NaCl followed by 0.2M sodium acetate pH 4.8. The small precipitate that formed was discarded (L. Kohn, personal communication). The proteins were then again precipitated with 60% saturated ammonium sulfate, dissolved in 0.1M acetic acid and dialyzed against 1mM sodium acetate pH 3.6, 6M urea.

The protein precipitated from 10 *l* of amniotic fluid was applied to a phosphocellulose column (2.5 × 20 cm) equilibrated with 1mM sodium acetate pH 3.6, 6M urea and eluted with a linear gradient (600/600 m*l*) from 0 to 0.3M NaCl. The effluent volume was monitored at 230 nm and collected into 15 - 20 m*l* fractions. Portions from the fractions were assayed with anti-pro α1 (I) antibodies for inhibiting activity as indicated below. Fractions containing serologically active material obtained from 3 runs were combined, dialyzed against 50mM Tris/HCl pH 8.6, 2M urea and applied to a column of DEAE-cellulose (2.5 × 20 cm) which was equilibrated with the same buffer. Elution of protein was carried out with a linear gradient (500/500 m*l*) from 0 to 0.3M NaCl. Again serologically reactive material was identified and fractions containing the activity were concentrated by ultrafiltration (Diaflow) and chromatographed on a Bio-Gel P-150 column (3 × 115 cm) in 0.1M Tris/HCl pH 7.4. The peak fractions obtained at this step were desalted on Bio-Gel P-2 in 0.1M formic acid and lyophilized. In some studies fractions from the P-150 column