Procollagen Biosynthesis in Embryonic Chick Arteries

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Collagen is initially synthesized as a precursor form called procollagen (for reviews, see Schofield & Prockop, 1973; Grant et al., 1974). By using freshly isolated cells, obtained by enzymic digestion of embryonic chick tendon (Dehm & Prockop, 1971), cartilage (Dehm & Prockop, 1973) and lens (Grant et al., 1972), it has been possible to study the synthesis and secretion of the different types of procollagen from each of these tissues. Here we report on the synthesis and secretion of procollagen by freshly isolated cells from the major arteries of chick embryos.

The major arteries of 17-day chick embryos were dissected (Murphy et al., 1972) and the cells were isolated essentially as described by Dehm & Prockop (1971, 1973); 5 x 10^6–7 x 10^6 cells were obtained/embryo. The cells were incubated in modified Krebs medium at a density of 1 x 10^7 cells/ml.

Continuous labelling of the freshly isolated cells with \(^{14}\)C-proline indicated that the synthesis of \(^{14}\)C-labelled polypeptides, the synthesis of non-diffusible peptide-bound hydroxy\(^{14}\)C-proline and the secretion of peptide-bound hydroxy\(^{14}\)C-proline were linear for up to 6h. In two separate experiments a lag of about 7min was observed between the time at which the incorporation of \(^{14}\)C into polypeptides became linear and the time at which the synthesis of peptide-bound hydroxy\(^{14}\)C-proline became linear (Fig. 1). This compares with a lag of 2–3min in both tendon and cartilage cells (Dehm & Prockop, 1972, 1973) and it may indicate that, in the artery cells, the synthesis of collagen hydroxyproline may be largely a post-ribosomal event, whereas in tendon and cartilage cells it occurs mainly on nascent chains (see Grant et al., 1974). There was also a considerable lag (about 31 min in 2expts.) between the time at which synthesis of peptide-bound hydroxy\(^{14}\)C-proline became linear and the time at which its secretion became linear (Fig. 1). This is similar to the lag of about 33 min seen in cartilage cells (Dehm & Prockop, 1973), but it contrasts with that of about 18 min seen in tendon cells (Dehm & Prockop, 1972). This observation is of interest since the artery cells appear to be synthesizing type I procollagen (see below), as do tendon cells, and the difference in lag times for these two cell types synthesizing the same type of procollagen may have important implications in terms of the intracellular processing of procollagen polypeptide before their secretion.

Chromatography of reduced \(^{14}\)C-labelled polypeptides from the medium on 6% agarose indicated that the bulk of the hydroxy\(^{14}\)C-proline was in pro \(\alpha\) chains of procollagen. When reduction was omitted, essentially all of the \(^{14}\)C-procollagen was eluted as a high-molecular-weight aggregate (>300000) in which the pro \(\alpha\) chains were apparently linked by interchain disulphide bonds. In a parallel experiment in which the medium \(^{14}\)C-labelled polypeptides were treated with pepsin at 15°C, the hydroxy\(^{14}\)C-proline-containing polypeptides were now eluted from the agarose column with collagen \(\alpha\) chains indicating that the procollagen was in a triple-helical conformation.

In experiments to determine what type of procollagen was being synthesized by the artery cells the hydroxy\(^{14}\)C-proline-containing polypeptides in the medium were first precipitated by using 30%-saturated (NH₄)₂SO₄ (Dehm & Prockop, 1973); about 90 % of the medium hydroxy\(^{14}\)C-proline was precipitated by this procedure. The precipitated \(^{14}\)C-labelled polypeptides were examined, after reduction, by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate, and two radioactive bands, corresponding to pro \(\alpha_1\) and pro \(\alpha_2\), were observed. The ratio of radioactivity in the pro \(\alpha_1\) band to that in the pro \(\alpha_2\) band was about 2 in several experiments, which is the value expected for a type I procollagen. Procollagen from the medium of tendon cells, which appears to be a type I procollagen as judged by these criteria (see Schofield et al., 1974), gave similar results to those observed for the artery procollagen when it was electrophoresed in parallel gels. These observations indicate that the artery cells from 17-day embryo synthesize and secrete a type I procollagen. This is somewhat surprising in view
Fig. 1. Time-course of incorporation of \([^{14}C]\)proline and synthesis and secretion of non-diffusible \([^{14}C]\)hydroxyproline by cells isolated from the arteries of 17-day chick embryos

Some \(8 \times 10^7\) cells were incubated in 16ml of Krebs II medium plus 10\% (w/v) foetal calf serum and samples (2.5ml) were withdrawn at the indicated times. ■, Total non-diffusible \(^{14}\)C in cells plus medium; ●, total non-diffusible hydroxy\(^{14}\)C proline in cells plus medium; ○, non-diffusible hydroxy\(^{14}\)C proline in the medium.

of recent reports that human aorta contains substantial amounts of type I and type III collagen (Chung & Miller, 1974; Trelstad, 1974). Had type III procollagen been present in the medium of the artery cells in significant amounts, this would have resulted in a pro \(\alpha 1\) : pro \(\alpha 2\) ratio significantly higher than 2.

In further experiments to determine whether type III procollagen might be synthesized at earlier stages of development of the chick embryo, the procollagen secreted by cells isolated from 10-day embryos was examined by gel electrophoresis. However, radioactive bands corresponding to pro \(\alpha 1\) and pro \(\alpha 2\) chains in a ratio of about 2 were again observed.

Thus, freshly isolated cells from the arteries of 17-day and 10-day chick embryos synthesize and secrete procollagen, which appears to be a type I procollagen. The rates of synthesis and secretion of type I procollagen by cells from the arteries of 17-day embryos appear to be considerably slower than the rates of synthesis and secretion of type I procollagen by tendon cells from 17-day embryos.

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The Isolation of Collagen-Associated Molecules from Bovine Nasal Cartilage

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The solubilization of non-collagenous molecules by collagenase digestion can be used as a criterion to establish the existence of aggregates between collagen and other macromolecules (Olsen et al., 1973; Wolff et al., 1971; Benya et al., 1973). We have used collagenase digestion to obtain the non-collagenous molecules associated with a preparation of bovine nasal cartilage collagen.

Bacterial collagenase (Clostridium histolyticum) from a commercially available crude preparation (P-L Biochemicals, Milwaukee, Wis., U.S.A.) was purified by ion-exchange chromatography on SP-Sephadex and DEAE-cellulose, followed by gel filtration on Sephadex G-200 (V. Lee-Own & J. C. Anderson, unpublished work). The end product had negligible non-specific proteolytic activity, when analysed by the method of Peterkofsky & Diegelmann (1971).

Bovine nasal cartilage collagen was prepared by two different methods. In the original method (A), shredded cartilage was exhaustively extracted with 0.15M- NaCl-0.02M-NaH₂PO₄, pH 7.2 (phosphate-buffered saline), followed by 3M-MgCl₂ solutions (Anderson & Jackson, 1972). In the second method (B) shredded cartilage was repeatedly extracted with 3M-MgCl₂ without the initial extractions by phosphate-buffered saline. Extracts from the 3M-MgCl₂ treatment were exhaustively dialysed, and the resulting precipitate was separated by centrifugation (2000g, 30min). The precipitates were extracted twice with phosphate-buffered saline to remove any readily soluble components. Very little material was extracted. The residues were analysed for hydroxyproline by the method of Woessner (1961). Residues obtained by methods A and B contained 4.4% and 3% hydroxyproline respectively, indicating that they had an appreciable collagen content (30-50%).

The residues (100mg) were suspended in 0.05M-Tris-0.005M-CaCl₂-0.02M-NaNO₃, pH 7.5 (9.8ml), and collagenase (200μg) was added. The mixture was incubated for 18h at 37°C. The negligible amount of undigested material was removed by centrifugation, and the soluble digest was dialysed and freeze-dried.

Chemical analysis of the digests (Table 1) showed that they contained hydroxyproline. However, although several distinct protein bands were observed on polyacrylamide-gel electrophoresis, none of these corresponded to the α, β, or γ chains of collagen (Fig. 1).

The digests were chromatographed on a Sepharose 4B column (38cm×2.5cm) in phosphate-buffered saline, at a flow rate of 12ml/h. Two peaks of absorption at 280nm were detected, one of which appeared in the void volume, and the other in the included volume. For both A and B digests, the excluded peak contained the bulk of the hexuronic