INFLUENCE OF EXTRACELLULAR PYROPHOSPHATE ON THE SYNTHESIS OF COLLAGEN BY CHONDROCYTES

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1. Introduction

The constituents of extracellular matrix play a major role in the structural and functional properties of tissues. Furthermore, alterations in the extracellular matrix can induce changes in metabolism and phenotypic expression of the cells. Type II collagen (composed of three α_1 (II) chains) is located specifically in cartilage, in close association with the proteoglycan aggregates. Nevertheless, once the extracellular matrix is disrupted, chondrocytes from the same tissue can synthesize different types of collagen molecules, leading to a disturbance in the macromolecular assembly. The cartilage undergoing osteoarthritic changes, as well as normal aged cartilage, produces type I collagen (composed of two α_1 (I) and one α_2 chains) in addition to the tissue-specific type II collagen [1–3]. Chick-embryonic chondrocytes, when subcultured to senescence, synthesize type I collagen and type I trimer rather than type II collagen [4]. The chondrocytes from normal rabbit-articular cartilage switch their phenotypic expression to type I collagen when isolated from the tissue and maintained in monolayer culture [5–7]. In suspension culture, these cells produce primarily type II collagen in the medium lacking $CaCl_2$ [5,7] and type I collagen in complete medium [5,8–10]. These findings suggest a crucial role of calcium ions in altered cell–matrix interactions and cellular biosynthesis, as their concentrations may vary in certain pathological and physiological conditions.

An increase in alkaline phosphatase activity and pyrophosphate concentration has been reported in the synovium and cartilage matrix involved in osteoarthritis [11,12]. The deposition of calcium pyrophosphate or hydroxyapatite crystals has also been observed in these tissues [13,14]. In this communication, we have described the effect of extracellular pyrophosphate on the biosynthesis of collagen by chondrocytes.

2. Materials and methods

The chondrocytes were obtained from the articular cartilage of knee and hip joints of rabbits (2 months) by enzymic digestion [5]. The cells were allowed to grow to confluency in monolayer cultures in Ham's F-12 nutrient mixture with 10% (v/v) fetal calf serum and antibiotics, and then transferred to suspension cultures in Dulbecco's modified Eagle's medium with the glucose concentration increased to 4.5 g/liter and $CaCl_2$ totally omitted. The medium contained fetal calf serum (10%), antibiotics and various concentrations of Na-pyrophosphate. After 48 h, Na-ascorbate (25 μg/ml), β-aminopropionitrile (50 μg/ml) and [2,3-^{3}H]proline (10 μCi/ml; spec. act. 24.5 Ci/mmol) were added to the medium. After 24 h incubation under these conditions, the medium was centrifuged and the cells extracted with 0.5 M acetic acid. To assess the type of collagen synthesized, the labelled collagen in the medium and cell extract was purified along with carrier rat-skin acid-soluble collagen and analyzed for the subunit composition by carboxymethyl cellulose (CMC) chromatography [5]. To confirm the identities of the α-chains, the fractions corresponding to each chain were pooled and treated with cyanogen bromide (CNBr). The peptides thus obtained were separated on a CMC column and compared with those of type I and II collagen [5].

Table 1
Synthesis of collagen by chondrocytes in suspension culture

Treatment	$\alpha_1 : \alpha_2$ chain ratio
None	> 20.0
Na-pyrophosphate	
1 mM	2.2
100 μM	2.9

The cells were maintained in suspension culture in medium with no $CaCl_2$, or the same medium with various concentrations of Na-pyrophosphate. $\alpha_1 : \alpha_2$ chain ratio indicates the relative proportions of types I and II collagen synthesized (chain ratio for type I collagen is 2.0). The results were confirmed by analysis of CNBr-peptides (fig.2). Each value represents the mean of 3 or more experimental analyses.

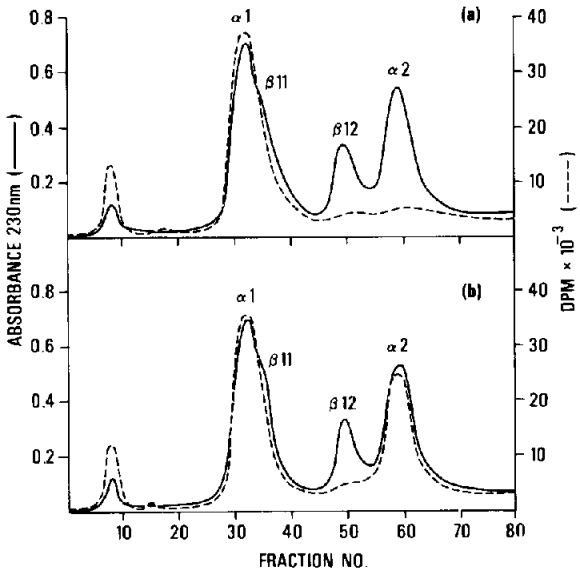

Fig.1. CMC elution patterns of collagen synthesized by chondrocytes. [^{3}H]Proline-labelled collagen was treated with pepsin at 4°C for 72 h, mixed with the carrier rat-skin acid-soluble collagen and purified by precipitation with 25% NaCl, at pH 7.5. The precipitate was dissolved in 0.06 M Na-acetate buffer, pH 4.8, and chromatographed on a CMC column (0.9 × 10 cm) at 40°C with a linear gradient from 0–0.1 M NaCl (80 ml each). Solid lines represent the elution pattern of carrier collagen; dotted lines represent that of labelled collagen synthesized by chondrocytes in: (a) medium without $CaCl_2$; (b) same medium in presence of 1 mM Na-pyrophosphate.

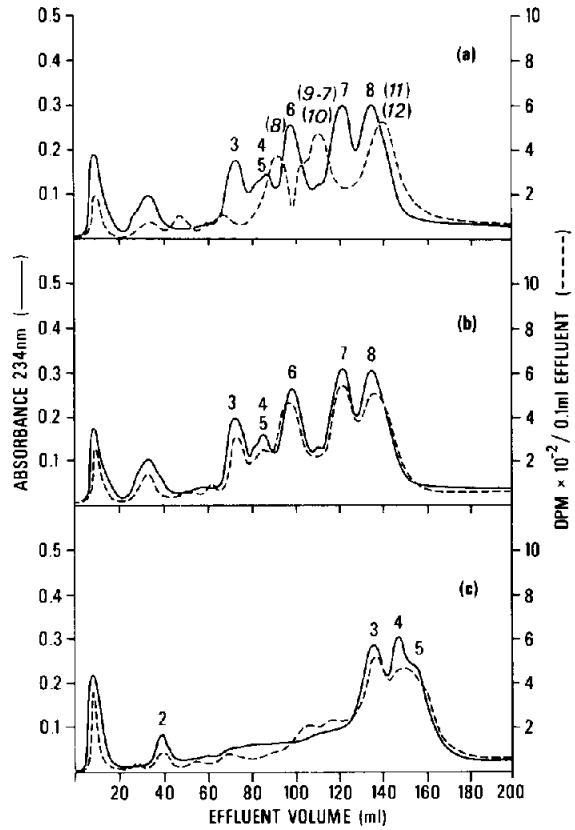

Fig.2. Elution patterns of CNBr-peptides of α_1 and α_2 chains. The CNBr-peptides were produced by treating the individual chains with excess CNBr. Lyophilized peptides were dissolved in 0.02 M Na-citrate buffer, pH 3.6, containing 0.02 M NaCl and applied to a CMC column (0.9 × 10 cm) at 40°C. The elution was carried out using the same buffer with a linear gradient from 0.02–0.14 M NaCl over total vol. 200 ml. (a) (———) Peptides of α_1 (I) chains of carrier collagen and (– – – –) peptides of α_1 (II) chains (denoted by the numbers in parentheses) synthesized by chondrocytes in the medium without $CaCl_2$. (b) (———) Peptides of α_1 (I) chains of carrier collagen and (– – – –) peptides of α_1 (I) chains synthesized by chondrocytes in the same medium in presence of Na-pyrophosphate. (c) (———) Peptides of α_2 chains of carrier collagen and (– – – –) peptides of α_2 chains of type I collagen synthesized in presence of Na-pyrophosphate.

3. Results and discussion

The data in table 1 and fig.1,2 indicate that the chondrocytes, maintained in suspension culture medium devoid of $CaCl_2$, produced primarily type II

collagen (fig.1A,2A), while the addition of Na-pyrophosphate to this medium allowed the cells to synthesize significant amounts of type I collagen, rather than type II. The ratio of labelled $\alpha_1 : \alpha_2$ chains was between 2.0 and 3.0 and these chains mainly showed the characteristic CNBr-peptide patterns of α_1 and α_2 chains of type I collagen (fig.2B,C).

The addition of excess pyrophosphate to suspension culture medium allowed the chondrocytes to change their phenotypic expression. Similar results have also been obtained with $CaCl_2$ [5], suggesting that the local increase in calcium or pyrophosphate ions around the chondrocytes stimulates them to produce type I collagen. The clones of chick embryonic chondrocytes switch their synthesis of collagen from type II to type I and type I trimer, as a result of aging in culture [4]. One chondrocyte, therefore, has the capability of synthesizing more than one type of collagen molecule. The nature of the product may depend upon various intracellular and extracellular factors.

As mentioned earlier, in pathological conditions such as chondrocalcinosis or some cases of osteoarthritis, the deposition of calcium pyrophosphate and hydroxyapatite crystals occurs in the synovial tissue and articular cartilage of involved joints. The smallest, and presumably the earliest, deposits are seen around the lacunae of chondrocytes in the articular cartilage [13,14]. Furthermore, osteoarthritic, but not normal, articular cartilage slices liberate pyrophosphate into the medium, when incubated in vitro [11,12]. Similar findings were observed with immature rabbit cartilage [11,12]. It was postulated that pyrophosphate produced by rapidly dividing chondrocytes in osteoarthritic cartilage may substantially account for its elevated levels in synovial fluid [11,12]. Additional sources of pyrophosphate in synovial fluid may be plasma and dissolution of bone mineral [13]. The origin of pyrophosphate in synovial fluid may not be chondrocytic [15]. Nevertheless, all these findings point out a local increase in pyrophosphate concentration around the chondrocytes, leading to a supersaturation level and formation of the crystals with calcium, in the lacunae and cartilage matrix. These altered extracellular conditions may cause a change in the cellular metabolism and phenotypic expression in vivo.

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