THE OCCURRENCE OF A NEW TYPE OF PROTEOCHONDROITIN SULFATE IN THE DEVELOPING CHICK EMBRYO

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1. Introduction

In the development of an embryonic chick limb, temporal and spatial transitions have been observed in the type of collagens. Undifferentiated mesodermal cells have been reported to synthesize Type I collagen, and Type II collagen appears in accordance with the onset of chondrogenesis in limb development [1]. With collagen, proteochondroitin sulfate is one of the major components of the extracellular matrix. Two types of proteochondroitin sulfate have been found in the cartilage of chick embryo, based on sucrose gradient separation [2]. The occurrence of proteochondroitin sulfates has not been reported, prior to chondrogenesis, only that of chondroitin sulfates have been observed [3–6].

We have been interested in identifying the type of proteochondroitin sulfate synthesized by the chick embryo prior to chondrogenesis. We report here that the chick embryo does synthesize a new type of proteochondroitin sulfate, before chondrogenesis takes place. It is distinctly different from two proteochondroitin sulfates, hitherto found in the embryonic chick cartilage, with respect to its sedimentation profile on sucrose gradient, amino acid composition and the length of mucopolysaccharide chain.

2. Materials and methods

Limb buds and axial organs (notochords, neural tubes and somites) were dissected, without aid of enzyme treatment, from 4 day old embryos (stage 23) and 3 day old embryos (stage 18), respectively. Vertebrae were dissected from 5 (stage 26) and 7 (stage 31) day old embryos. Proteochondroitin sulfates were extracted from these tissues under dissociative conditions [7]. These samples were then purified by sedimentation on sucrose gradient [2]. Two types of proteochondroitin sulfate from the epiphyseal cartilages of 13 day old embryos were prepared by the same method, yielding a faster (PCS-H) and a slower sedimenting proteochondroitin sulfate (PCS-L). Gel filtration on Sepharose 6B (Pharmacia) and amino acid analyses were carried out on the samples before and after the alkaline treatment. The relative contents of 4- and 6-sulfated disaccharides were estimated by the procedure of Saito et al. in the chondroitinase ABC (Seikagaku Kogyo) digest of proteochondroitin sulfates [8].

3. Results and discussion

In the limb buds of 4 day old embryos, only one peak was detected occupying the position between PCS-H and PCS-L (fig.1). Judging from the data to be shown below, the peak was identified as that of a proteochondroitin sulfate. This newly proteochondroitin sulfate will be referred to PCS-M in the present report. In notochords, neural tubes and somites of 3 day old embryos, their main peaks also corresponded to PCS-M (fig.2). Furthermore PCS-M was detected in the area pellucida of an embryo at stage 8 [9]. In the cartilage forming tissues, such as vertebrae (fig.3, see also fig.2C) and limbs [10], PCS-M was replaced with PCS-H as mesodermal cells differentiated into

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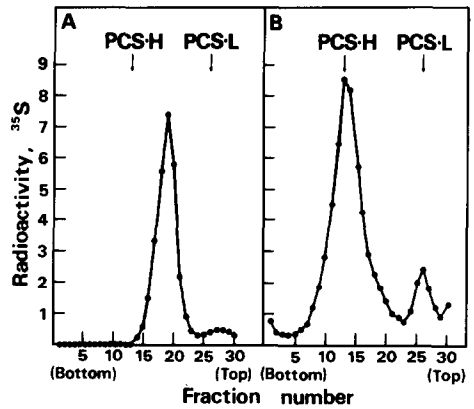

Fig.1. Sedimentation profiles in sucrose gradient of 4 day old limb buds (A) and 13 day old epiphyses (B). Abscissa, fraction number; ordinate, arbitrary unit of radioactivity. Limb buds were incubated for 5 h at 37°C with a liquid nutrient medium consisting of Hanks solution, fetal calf serum (GIBCO), and F12 medium, 2:2:1. 20 μCi/ml of [^{35}S] H_2SO_4 was used to label proteochondroitin sulfates. The proteochondroitin sulfates were extracted at 4°C in 4 M guanidinium chloride–50 mM Tris–HCl buffer containing 0.1 M ε-aminocaproic acid and 0.01 M EDTA, pH 7.0, for 48 h. Solid CsCl was added to the extracts to give a density of 1.50 g/ml and a density gradient was formed by centrifugation at 40 000 rpm for 44 h at 22°C (in a Beckman Type 50 Titanium Rotor). The bottom fractions ($\rho > 1.53$) were pooled, in which approx. 70% of radioactivity was recovered. Samples were dialyzed against the extracting solution and condensed by a Collodion bag (Sartorius-Membrane filter). The sedimentation on 5–20% sucrose gradient formed in the presence of 4 M guanidinium chloride was carried out at 24 000 rpm for 38.5 h at 2°C (in a Beckman Type SW27.1 Swinging-Bucket Aluminium Rotor).

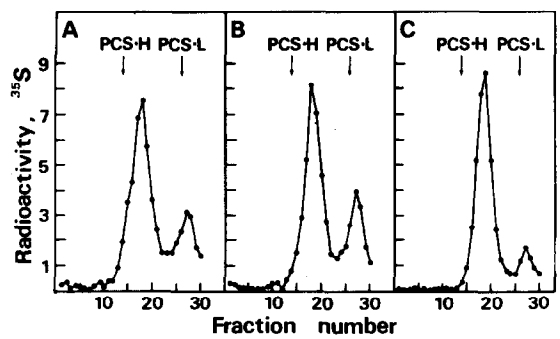

Fig.2. Sedimentation profiles in sucrose gradient of notochords (A), neural tubes (B) and somites (C) of 3 day old embryos. Abscissa, fraction number; ordinate, arbitrary unit of radioactivity.

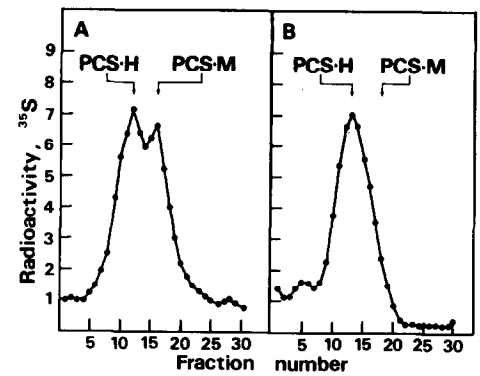

Fig.3. Sedimentation profiles in sucrose gradient of vertebrae of 5 day (A) and 7 day old embryos (B). Abscissa, fraction number; ordinate, arbitrary unit of radioactivity.

chondrogenic cells. The same replacement, from PCS-M to PCS-H, was observed in the development of chick notochord [11]. The small peak found above PCS-M, shown in fig.1A and 2, will be discussed later.

Characterization was carried out on PCS-M which was purified from limb buds of 4 day old embryos. The composition of PCS-M is shown in table 1 as compared with that of PCS-H and PCS-L. Amino acid composition of PCS-M was different from that of the others. It is characteristic that the threonine content of PCS-M relatively high. Hexosamine contents of PCS-M were lower than those of the others. The relative amounts of isomeric chondroitin sulfates and chondroitinase ABC resistant were the following: chondroitin 6-sulfate, 63.3%; chondroitin 4-sulfate, 34.4%; chondroitinase ABC resistant, 2.3%.

Three types of proteochondroitin sulfate were excluded on a Sepharose 6B column. To estimate length of their mucopolysaccharide chains, alkaline β-elimination was carried out. The elution profiles on the Sepharose 6B column are shown in fig.4. The mucopolysaccharide chain of PCS-M was longer (K_{av} = 0.24) than that of PCS-H (K_{av} = 0.44) and similar to that of PCS-L (K_{av} = 0.27). The content of amino acids destroyed by alkaline β-elimination is also shown in table 1. It is interesting that the ratio of alkali-labile threonine residues to original threonine residues in PCS-M is very high in contrast to other proteochondroitin sulfates.

From the data shown above, it has been clearly shown that a proteochondroitin sulfate, PCS-M, is

Table 1
Amino acid and hexosamine compositions of PCS-M, PCS-H and PCS-L

	PCS-M	PCS-H	PCS-L
Aspartic acid	88	54	107
Threonine	114 (31)	91 (16)	77 (3)
Serine	112 (27)	129 (61)	94 (20)
Glutamic acid	137	165	138
Proline	68	60	76
Glycine	70	111	65
Alanine	53	71	54
Valine	75	66	54
Methionine	23	8	12
Isoleucine	42	50	59
Leucine	62	55	102
Tyrosine	25	16	29
Phenylalanine	35	36	30
Lysine	45	33	40
Histidine	23	28	19
Arginine	28	27	44
Glucosamine	34	70	51
Galactosamine	612	2872	1052

Amino acid contents are expressed as residues per 1000 residues. Cystine was found to be less than 5 residues. Figures in parentheses at threonine and serine show the contents of the amino acids destroyed by alkaline β-elimination. Alkaline β-elimination was carried out in 0.5 M NaOH for 40 h at 4°C. Hexosamine contents are expressed as residues per 1000 amino acid residues.

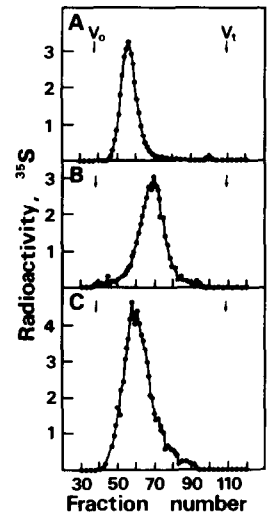

Fig.4. Elution profiles of the mucopolysaccharide chains of PCS-M (A), PCS-H (B), and PCS-L (C) chromatographed on a Sepharose 6B column. The samples were obtained by alkaline β-elimination of each proteochondroitin sulfate. They were eluted with 2 M guanidinium chloride–50 mM Tris–HCl buffer, pH 7.2. Abscissa, fraction number; ordinate, arbitrary unit of radioactivity.

synthesized by the limb buds of 4 day old chick embryos and by other embryonic tissues so far examined, and that gross structure of PCS-M definitely differed from that of PCS-H and PCS-L.

Palmoski et al. separated proteochondroitin sulfates, produced by cultured chondrocytes from 13 day old chick embryos, into two proteochondroitin sulfate fractions on a column of Bio-Gel A-150m [12]. They postulated the large molecular proteochondroitin sulfate to be cartilage specific while the small one to be ubiquitous proteochondroitin sulfate. On our sucrose gradients, it was found that the large molecule corresponded exactly to our PCS-H and the small one to PCS-L. Contrary to their proposal, we were unable to detect PCS-L in any tissues examined before chondrogenesis initiated. We found, instead, a new type of proteochondroitin sulfate, PCS-M, as already described.

On sucrose gradients, the appearance of a small peak above that of PCS-M was observed (fig.1A and 2). It was different from PCS-L, because it sedimented more slowly than PCS-L. It was further confirmed by gel filtration that the material of this peak was not PCS-L. Further characterization has not been made.

The transition in the type of proteochondroitin sulfate, from PCS-M to PCS-H, was found in accordance with the onset of chondrogenesis. This may suggest the switching of genes in function, from mesodermal specific to cartilage specific, for extracellular matrix components as chondrogenesis undergoes. This idea may also be supported by the reported occurrence of a collagen type shift during chondrogenesis [1].

Recently the existence of three types of proteochondroitin sulfate was reported in a culture system of several embryonic cells [13,14]; one of them (peak 3) may correspond to our PCS-M.

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