BIOSYNTHESIS OF CHONDROITIN SULFATE PROTEINS. PULSE LABELING EXPERIMENTS WITH RADIOSULFATE OF FOUR POOLS OF CHONDROITIN SULFATE PROTEINS IN CALF RIB CARTILAGE

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Received 11 January 1974

1. Introduction

In calf rib cartilage proteoglycans mainly consisting of chondroitin-4-sulfate proteins (Ch-4-S-P) [1] exhibit considerable chemical and metabolic heterogeneity of their molecules [1, 2] as well as different binding qualities in the tissue [3, 4]. Careful extraction with solutions of guanidine of different molarity yields four fractions of chemically and metabolically heterogeneous proteoglycans [5–8]. After labeling with radiosulfate during increasing incubation periods one of these fractions (the insoluble one) showed the highest specific radioactivity of sulfate esters compared to that of the three soluble ones. Therefore, this insoluble proteoglycan fraction was proposed as a priming pool for proteoglycans feeding the three soluble pools with de novo synthesized chemically and metabolically heterogeneous proteoglycans [7, 9]. Pulse chase experiments with radiosulfate have to be done to proof this hypothesis.

2. Experimental

Details for preparation and incubation of cartilage slices [2, 10] as well as the extraction procedure with solutions of guanidin of different molarity [7] were previously described. In brief, three parts (A, B, C, each of 4 g) were incubated (each two portions) for 20 min at 37°C with 320 μCi of carrier free $^{35}SO_4^{2-}$ in a Warburg apparatus under anaerobic conditions (95% N_2, 5% CO_2). To control metabolic activity of cartilage cells the production of CO_2 was manometrically measured during the incubation time [10]. Slices and medium were immediately cooled in an ice-water bath at the end of the incubation.

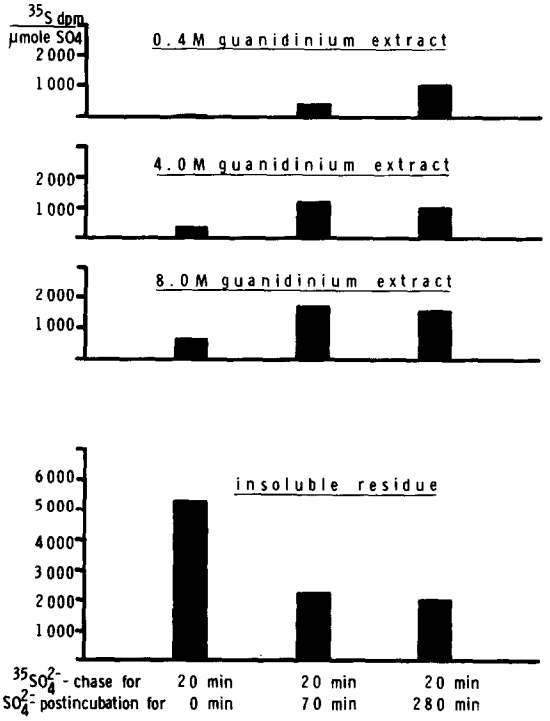

Fig. 1. ^{35}S-labeling of 4 pools of proteoglycans in calf rib cartilage by 'chasing' experiments with radiosulfate. For further details see text.

Slices and medium of part A were made 0.4 M with

Cartilage fractions	Macromolecular-bound radiosulfate after					
	20 min 'chase' with radiosulfate		70 min Postincubation with cold sulfate		280 min Postincubation with cold sulfate	
	dpm/g w.w.	% of Total	dpm/g w.w.	% of Total	dpm/g w.w.	% of Total
extract I	540	0.5%	3 520	3%	8 870	8%
extract II	21 560	20%	68 500	64%	68 000	62%
extract III	3 830	4%	7 400	7%	6 920	6%
Insoluble residue	80 130	75%	27 720	26%	25 420	23%
Total	106 060	100%	107 140	100%	109 210	100%

Fig. 2. Distribution pattern of macromolecular-bound radiosulfate in cartilage fractions after some radioactive and non-radioactive 'chases'. For further details see text.

respect to guanidinium chloride and extracted for 24 hr at pH 5.8 by gently stirring in an ice-bath (*Extract I*). The extraction procedure was continued by applying 4.0 M guanidine, pH 5.8 at 25°C and then 8.0 M guanidine, pH 5,8 at 60°C, yielding *Extract II* and *III*, respectively. From the *insoluble residue* a chondroitin sulfate (ChS-) peptide fraction was prepared by digestion with papain and purified by precipitation with cetylpyridinium chloride [7].

Slices of part B and C were separated from their medium, washed four times with 20 ml ice-cold medium containing 0.5 mM SO_4^{2-}, dried between filter papers, then washed and dried once again. All these procedures were done at 4°C. Medium and washings contained less than 5% of total uronic acid and were discarded. Finally the slices were postincubated at 37°C in 54 ml medium containing 0.5 mM cold sulfate for 70 and 280 min resp.. At the end of this incubation slices and medium were treated as discribed for part A.

Proteoglycans of extract I and II were purified by applying CsCl gradient centriguation under associative and dissociative conditions (cf. [3]). From this purified chroitin sulphate protein (ChS-P) of extract II and the proteoglycans of extract III ChS-peptide fractions were prepared by digestion with polymer-bound papain as previously described [7]. ChS-peptide fractions of proteoglycans of extract II and III as well as of the insoluble residue were fractionated on Ecteola cellulose according to increasing chain length and degree of sulfation [1, 4, 10]. Analysis of the components, ChS chain length and determination of absolute radioactivity (dpm) were carried out as described previously [7].

3. Results and discussion

After the extraction procedure the distribution of uronate was 16% in the insoluble residue, 11% in extract I, 69% in extract II and 4% in extract III [6–8]. After a 20 min 'chase' with radiosulfate the highest labeling rate of sulfate ester groups is found in the ChS-peptide fraction of the insoluble residue (fig. 1): Specific radioactivity of sulfate esters of the insoluble residue to extract I, II and III is 54:1:3.5:7.0 respectively. During a postincubation period with cold sulfate for 70 or 280 min specific radioactivity of sulfate esters in the ChS-peptide fraction of the insoluble residue decreases to 43% and 40% respectively, whereas at the same time labeling rates of sulfate esters in proteoglycans of extract I increase 4.5 and 10.6 times, in those of extract II 3.5 and 3.0 times and in those of extract III 2.5 and 2.2 times respectively (fig. 1). These findings again indicate different labeling patterns of proteoglycans in extract I, II and III (cf. [5–9]). This also holds true during non-radioactive 'chasing' periods: However, the labeling rate of sulfate esters in the proteoglycans of the 3 extracts increases inversely proportional to that found after a 20 min radioactive 'chase'. These results could indicate differ-

Incubation time	Rate of CO_2 production	
	μmoles g^{-1} w.w.	μmoles h^{-1} g^{-1} w.w.
20 min $n = 6$	0.775 ± 0.169	2.375 ± 0.428
70 min $n = 2$	2.626 ± 0.217	2.251 ± 0.186
280 min $n = 2$	9.120 ± 0.371	1.954 ± 0.079

Fig. 3. Metabolic activity of cartilage slices during different radioactive and non-radioactive 'chases'. Mean values with standard deviation, for details see the experimental part.

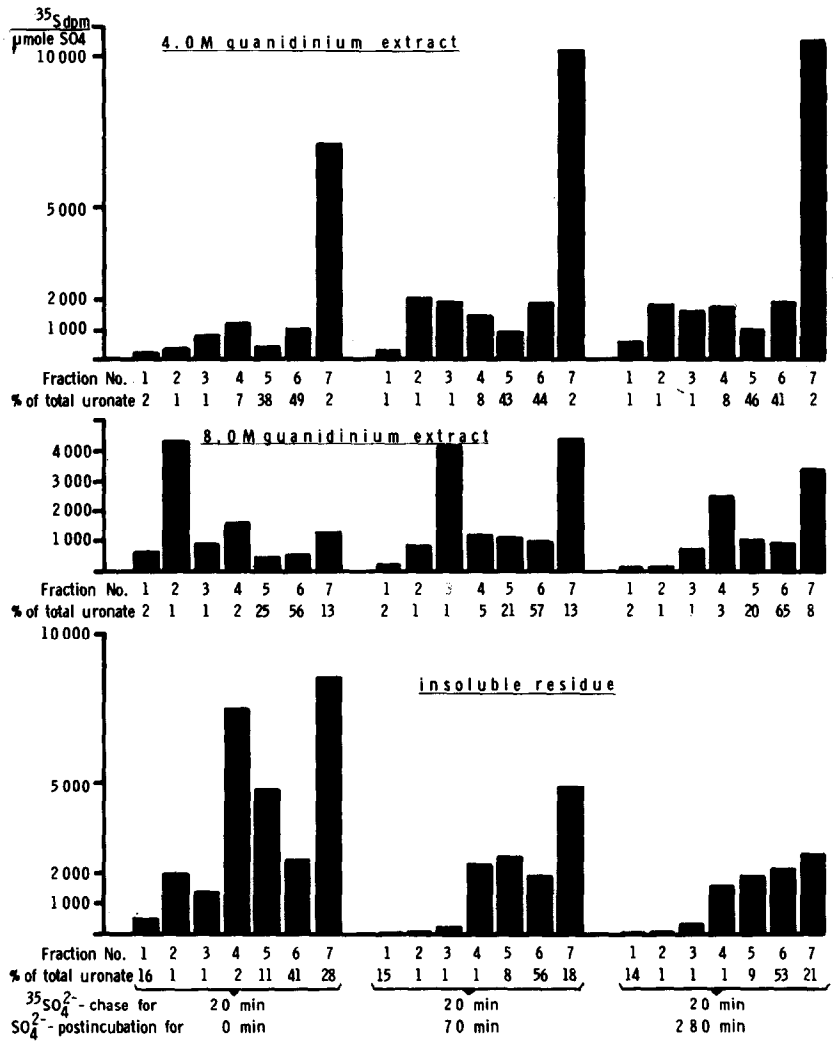

Fig. 4. ^{35}S-labeling of chondroitin sulfate side chains of proteoglycans in extract II and III and from insoluble residue by 'pulse-chase' experiments in calf rib cartilage. Proteoglycans from extract II were purified by CsCl density gradient centrifugations splitting off at least 6 small mostly lower labeled proteoglycan fractions. In general ChS side chains were isolated by papain digestion and fractionated on Ecteola-cellulose. For further details see text.

ent interrelations between the localization of these three proteoglycan pools to the priming pool.

In addition, these findings indicate the transfer of macromolecular-bound radiosulfate from the insoluble residue to the three soluble fractions of the cartilage as it is clearly demonstrated in fig. 2. After the 20 min radioactive 'chase' the amount of total incorporated ^{35}S dpm per 1 g cartilage remains constant during the following non-radioactive 'chases', thus demonstrating a complete in vitro-system in the cartilage slices. This transfer of de novo synthesized proteoglycans from the insoluble residue into the three soluble fractions is not caused by dying processes of cartilage cells since metabolic activity of these cells does not show significant differences in the amount of liberated CO_2 during incubation times of 20, 70 and 280 min (see second column of fig. 3).

These findings of a transfer of de novo synthesized proteoglycans can be further supported (fig. 4): After the separation of ChS-peptide fractions from the

purified ChS-P in extract II, from the proteoglycans in extract III and from the insoluble residue on Ecteola-cellulose 7 ChS side chain fractions are obtained exhibiting considerable differences in sulfate ester labeling besides increasing chain length and degree of sulfation [7, 11]. In general a characteristic labeling pattern is found: Shorter ChS side chains of the small fractions 2–4 are higher labeled than longer side chains of the bulk fractions 5 and 6. However, the longest ChS side chains of the small fraction 7 exhibit highest labeling rate of all 7 fractions (fig. 4). These findings indicate that smaller ChS side chain pools are faster labeled than larger ones, thus confirming earlier results [1, 2, 7, 10].

After a 20 min chase with radiosulfate nearly all ChS side chains of the insoluble proteoglycan fraction show higher labeling rates compared to that of the soluble proteoglycans of extract II and III. After a non-radioactive 'chase' of 70 min specific radioactivity of sulfate ester groups of all ChS side chains in the insoluble proteoglycan fraction decreases whereas that in the soluble proteoglycans increases generally keeping the above described labeling pattern. After a post-incubation period of 280 min this is held true only for ChS side chains of the purified proteoglycan from extract II and in a less way for proteoglycans from extract III. Labeling rates of the sulfate esters in insoluble proteoglycans decrease strongly exhibiting nearly zero-values in short ChS side chain fractions 1–3. These findings indicate de novo synthesis of ChS-P in the insoluble priming pool which are labeled with cold sulfate during the non-radioactive 'chase' period. Some of these molecules are obviously transferred to proteoglycans of extract III.

In summary, the 'pulse chase' experiments with radiosulfate presented here evidence the transfer of de novo synthesized metabolically and chemically heterogeneous ChS-P of an insoluble priming pool into three differently soluble, metabolically and chemically heterogeneous proteoglycan pools of the calf rib cartilage.

Acknowledgement

This work was supported by grant Kl 193/6 from the Deutsche Forschungsgemeinschaft.

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