THE INHIBITION OF CHONDROITIN SULPHATE PROTEIN SYNTHESIS BY CYCLOHEXIMIDE

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1. Introduction

Chondroitin sulphate in cartilage is covalently linked to a protein core [1]. Studies on the incorporation of isotopically labelled precursors of the carbohydrate and protein moieties show that both are synthesized at the same time in cartilage preparations incubated *in vitro* [2]. Puromycin when added to cartilage cell suspensions inhibits both the incorporation of ^{14}C-serine into core protein and ^{14}C-acetate into the acid mucopolysaccharide moiety [3]. However, puromycin does not appear to inhibit the formation of uridine diphospho (UDP) *N*-acetyl-galactosamine, an intermediate in the formation of chondroitin sulphate, nor does it inhibit the transfer of *N*-acetyl-galactosamine from UDP-*N*-acetyl-galactosamine-^{3}H into chondroitin sulphate catalysed by a cell-free system. It has been suggested that the synthesis of the core protein therefore controls the synthesis of the entire protein polysaccharide molecule [3]. Cycloheximide, like puromycin, is an inhibitor of protein synthesis and functions at the ribosome level [4,5]. It has little effect on existing enzyme systems concerned in free amino acid or keto acid formation [6]. It has, however, been shown to inhibit the incorporation of ^{3}H-acetate and ^{14}C-serine into hyaluronic acid and dermatan sulphate in tissue culture [7] and of ^{14}C-glucose and ^{14}C-leucine into the protein and polysaccharide moieties of cartilage chondroitin sulphate protein *in vitro* [8]. If the synthesis of core protein precedes the synthesis of the polysaccharide moiety it should be possible to demonstrate a difference in the labelling of these two components immediately following the addition of cycloheximide. This study was undetaken to examine this possibility.

Abbreviations:

UDP *N*-acetyl-galactosamine	Uridine diphospho *N*-acetyl-galactosamine
CS-peptide	Chondroitin sulphate peptide
CTAB	Cetyl-trimethyl-ammonium bromide

2. Experimental

Slices of cartilage from the nasal septum of foetal calves were incubated with either ^{14}C-glycine or ^{14}C-glucosamine under the conditions described in the legend to fig. 1. After incubation the samples were boiled for 5 min to stop further incorporation and digested with papain, exhaustively dialysed against distilled water and the chondroitin sulphate attached to residual peptide (CS-peptide) precipitated with 1% CTAB. CS-peptide was recovered from the washed precipitate by treatment with ethanolic KCNS as described previously [9]. This preparation contained 90–95% of the tissue chondroitin sulphate and 50% of the glycine present in chondroitin sulphate-protein. The CS-peptide preparations were assayed for uronic acid [10] and those obtained from tissues incubated with ^{14}C-glucosamine were counted directly in a Nuclear Chicago Unilux scintillation counter. The radioactivity present in the galactosamine moiety of the CS-peptide (isolated as the dinitrophenol derivative) accounted for 80% of the total radioactivity of the CS-peptide fraction.

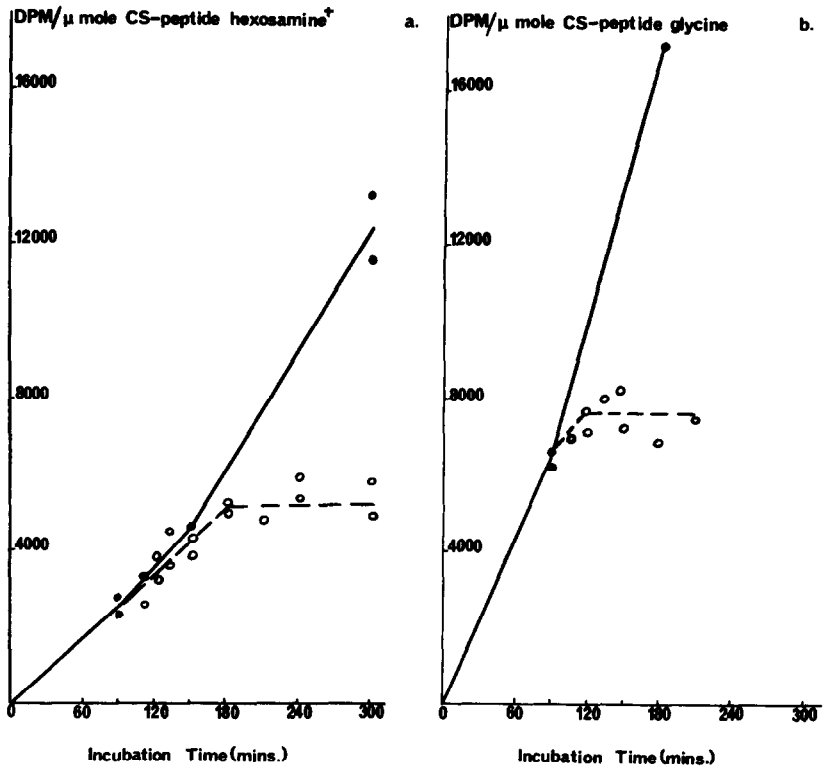

Fig. 1. Time course for the inhibition of ^{14}C-glucosamine (a) and ^{14}C-glycine (b) by cycloheximide. The conditions for incubation were as follows: 180–200 mg wet weight of sliced foetal cartilage (0.2–0.5 mm thickness) were incubated in 3 ml Krebs-Ringer bicarbonate medium pH 7.4 containing 2 mg glucose and 5.8 mg D-L-glutamine. The flasks, in duplicate, were incubated at 37°C and gassed with 95% oxygen and 5% CO_2 at 3 litres per minute. Slices were preincubated for 30 min in this medium and then transferred to new medium containing either 2 μc 1-^{14}C-D-glucosamine (Sp. Act. 4.05 mc/mM) or 4 μc 2-^{14}C-glycine (Sp. Act. 21.8 mc/mM). After 90 min incubation in this medium cycloheximide (100 μg) was added to each flask and duplicate flasks removed at various times. ●—● control points. ○--○ cycloheximide added.
* The hexosamine was calculated from the uronic acid value.

CS-peptide obtained from tissues incubated with ^{14}C-glycine were hydrolysed in 6 N HCl for 18 hr at 110°C and after removal of the HCl, the glycine was isolated on a Beckman Spinco Amino Acid Analyser fitted with a stream splitting device. Twenty five percent of the fraction was analysed with ninhydrin whilst the remaining fraction was assayed for radioactivity.

3. Results and discussion

Fig. 1 shows the time course of incorporation of ^{14}C-glucosamine and ^{14}C-glycine into the polysaccharide and peptide fractions of the cartilage CS-peptide and the effect of adding cycloheximide 90 min after beginning the incubation.

The incorporation of ^{14}C-glucosamine into the polysaccharide and ^{14}C-glycine into the peptide fractions of the CS-peptide is approximately linear for 5 hr (fig. 1a and b). However, after addition of cycloheximide, the incorporation of ^{14}C-glycine into core protein stops within 30 min (fig. 1b) whereas incorporation of ^{14}C-glucosamine into the chondroitin sulphate proceeds at the same rate for a further 60 min (fig. 1a). These results indicate that cycloheximide has little immediate effect on the mechanisms of chondroitin sulphate synthesis whereas it rapidly inhibits the synthesis of the protein core. The inhibition of chondroitin sulphate synthesis occurring 60 min

after the incorporation of ^{14}C-glycine into core protein has ceased may be due to a lack of core protein.

Thus the incorporation of ^{14}C-glucosamine into the chondroitin sulphate during this period is most likely due to the completion of existing chondroitin sulphate protein molecules rather than the initiation of new molecules. This result is consistent with the suggestion that the synthesis of core protein controls the synthesis of chondroitin sulphate protein [3].

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