## Chondroitin sulfate B metabolism

Relatively little information is available regarding the detailed mechanisms involved in the biosynthesis of the connective-tissue mucopolysaccharides. Studies performed in several laboratories have implicated uridine nucleotides as intermediates in these transformations[1,2]. Although available evidence indicates that the acid polysaccharides in skin (chondroitin sulfate B and hyaluronic acid) are in a dynamic metabolic state[3], the mechanism of breakdown of these polymers is not understood. The present preliminary report describes the behavior of chondroitin sulfate B in the presence of extracts of rabbit- or chick-embryo skin.

Chondroitin sulfate B labeled with $^{35}S$ in the sulfate group was prepared from rabbit skin obtained from animals injected with 500 $\mu$C of $^{35}SO_4^{=}$ 48 h prior to sacrifice. The polysaccharide was purified by conventional methods and exhibited satisfactory analyses[4]. Extracts were prepared from 2-week-old rabbit skin, or 21-day-old chick-embryo skin by homogenizing in a Waring Blendor for 2 min with 5 vol. 0.15 *M* KCl containing $5 \cdot 10^{-4}$ *M* glutathione. The extracts were centrifuged for 15 min at 18,000 $\times$ *g* and the supernatant solution treated with 0.1 vol. 2 % protamine sulfate. After standing for 15 min in an ice bath, the precipitate was removed by centrifugation and discarded. The protamine supernatant, 3–5 mg protein/ml, was used for the experiments described.

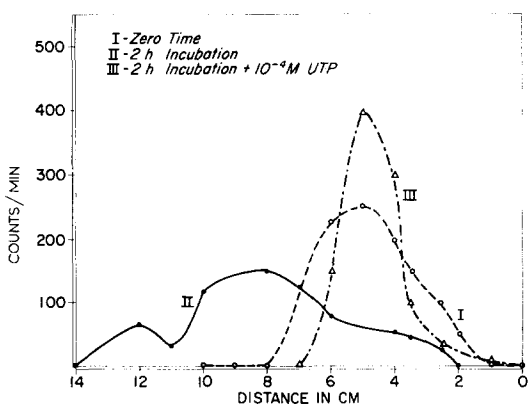


Fig. 1. Paper electrophoresis of chondroitin sulfate B incubations. Paper-electrophoretic patterns of incubation mixture containing [$^{35}S$]CSB, 1.0 $\mu$mole, 70,000 counts/min/mg; phosphate buffer (pH 7.5), 50 $\mu$moles; EDTA, 0.5 $\mu$mole; enzyme protein, 0.4 mg. After incubation for 2 h, the protein was removed by shaking with $CHCl_3$–butanol (9:1) and centrifugation. The polysaccharide material was precipitated from the supernatant by the addition of 2 vol. 95 % ethanol containing 0.5 *N* acetic acid. The precipitate was harvested by centrifugation, dissolved in 1.0 *M* $K_2CO_3$ and the resulting solution subjected to paper electrophoresis at 500 V for 45 min in 0.1 *M* acetate buffer, pH 5.0. When migration was complete, the strips were dried, sectioned and assayed for radioactivity in a Tri-Carb liquid scintillation counter (Packard Instrument Co., La Grange, Ill.) with toluene as solvent and PPO:POPOP (40:1) as scintillators. This method is desirable because of high efficiency (40 %) and elimination of self-absorption corrections. Since the compounds studied are not soluble in toluene, subsequent chemical assay for uronic acid content of eluted material was also performed and showed patterns coincident with radioactivity.

Abbreviations: ATP, adenosine 5-'triphosphate; GTP, guanosine 5'-triphosphate; CTP, cytosine 5'-triphosphate; UTP, uridine 5'-triphosphate; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2-(5-phenyloxazolyl)-benzene; CSB, chondroitin sulfate B; EDTA, ethylenediaminetetraacetic acid.

Incubation of choindroitin sulfate B with rabbit-skin enzyme followed by paper electrophoresis led to the pattern illustrated in Fig. 1. Degradation of the polysaccharide to a mixture of lower-molecular-weight fragments is indicated by the appearance of variable amounts of more rapidly migrating components. In contrast, when the incubation was performed in the presence of UTP, the polysaccharide fraction appeared to be of high molecular weight and less polydisperse than the starting material.

The UTP effect is highly reproducible and the nucleotide cannot be replaced by ATP or GTP in comparable concentration. UTP also appears to stimulate sulfate incorporation in a nucleotide-specific manner[5]. The effect of UTP on counts recoverable by ethanol precipitation is indicated in Table I.

Although the paper-electrophoretic patterns indicated the sulfate group to be still bound to an oligosaccharide unit, extracts were examined for chondrosulfatase activity (Table II). None were detected.

TABLE I

RECOVERY OF CHONDROITIN SULFATE B BY ETHANOL PRECIPITATION

Conditions as described in Fig. 1. After completion of incubation and removal of protein, sufficient ethanol–acetic acid was added to precipitate 50% of the initial polysaccharide. The resulting precipitate was harvested and assayed for radioactivity in a gas flow counter.

| | *Zero time* | *2-h incubation* | *2 h + $10^{-4}$ M UTP* |
|---|---|---|---|
| Crude extract | 1847 | 1284 | 1787 |
| Protamine supernatant | 1855 | 826 | 1696 |

TABLE II

CHONDROSULFATASE ACTIVITY OF SKIN EXTRACTS

Incubation mixture contained imidazole (pH 7.0), 100 $\mu$moles; EDTA, 2 $\mu$moles; [$^{32}$S]CSB, 4 $\mu$moles; [$^{35}$S]CSB, $2.6 \cdot 10^6$ counts/min protein, 1.3, 2.6 mg. At time *t*, 100 $\mu$moles $K_2SO_4$ and excess $BaCl_2$ were added. The $BaSO_4$ was extensively washed and counted.

| | *I* | *II* |
|---|---|---|
| Zero time | 152 | 319 |
| 2-h incubation | 146 | 327 |

In general, activities of chick-embryo-skin extract and rabbit-skin extract were similar. Comparable transformations were not observed with chondroitin sulfate A (cartilage) as substrate for the skin extracts. The nature of the intermediate products is under investigation.

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