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Effect of chondroitin on the uptake of radioactive sulphate into chondroitin sulphate

D'ABRAMO AND LIPMANN[1] have found that adenosine-3'-phosphate-5'-phosphosulphate (PAPS) is formed by an enzyme system present in particle-free extracts of chick-embryo condyles. By using ^{35}S-labelled PAPS they showed that the sulphate group was incorporated into chondroitin sulphate (CSA). A synthesis of CSA *de novo* from the component parts was demonstrated by the incorporation of radioactive acetate into CSA by this extract. The stage at which sulphation occurs in the biosynthesis of CSA is as yet unknown. MEYER and his co-workers[2,3] have suggested that the polysaccharide is formed first and then sulphated. This hypothesis was based on the isolation from cornea of the sulphate-free polysaccharide chondroitin[2], which they suggested was the immediate precursor of CSA. It was obviously of interest, then, to investigate the effect of added chondroitin on the biosynthesis of CSA.

CSA was isolated from bovine trachea by the method of BERA, FOSTER AND STACEY[4] and further purified by treatment with Sevag's Reagent and fractionation with alcohol from calcium acetate buffer, pH 5. The fraction precipitated by 30% (v/v) alcohol was removed and the fraction precipitated by 40% (v/v) alcohol was converted to the CETAVLON salt[6], the latter dissolved in 10% KCl and the free polysaccharide precipitated with alcohol. This was dissolved in water, dialysed, the solution passed through a column of Dowex 50, K form, and lyophilised (Found: N, 2.11; S, 5.03. Calc. for $C_{14}H_{19}NSO_{14}K_2 \cdot 4H_2O$: N, 2.3; S, 5.3). Chondroitin (K salt) was prepared from this material by the desulphation method of KANTOR AND SCHUBERT[5]. (Found: N, 2.73; S, 0.31. Calc. for $C_{14}H_{20}NO_{11}K \cdot 2H_2O$: N, 3.1; S, 0). Metachromasia was virtually absent when solutions of the product were tested with methylene blue[5] by measuring the absorption at 665 mμ.

The enzyme used in the study was a high-speed supernatant prepared from condyles of 15-day-old chick embryos by extraction with 0.01 *M* phosphate saline (1 ml per embryo)[1]. PAPS was prepared by incubation of adenosine triphosphate (ATP), $MgCl_2$, enzyme and tris(hydroxymethyl)aminomethane(Tris), pH 8.2, with

$Na_2{}^{35}SO_4$ (carrier free), in the proportions given in Table I. After incubation for 2 h at 37°, the solution was heated for 1 min at 100°, diluted with water and the nucleotides adsorbed on charcoal. After washing twice with 0.1 *M* Na_2SO_4, the nucleotides were eluted with pyridine, and dried in an air stream. The residue was dissolved in water and applied as a streak to filter paper (Schleicher and Schull 2043 B) and subjected to electrophoresis in 0.025 *M* citrate buffer, pH 5.5, at 2.7 V/cm for 16 h. The position of the u.v.-absorbing zones were determined by a u.v. photographic print and the radioactive zones located and measured with an automatic scanner. The PAPS band, travelling just in front of ATP, was eluted with water, adjusted to pH 8 with ammonia, concentrated, and again subjected to electrophoresis. PAPS was eluted with water, the pH adjusted to 8 with ammonia, and the solution kept frozen until used.

Results of the effect of added chondroitin on the incorporation of radioactivity from $^{35}SO_4^{=}$, [^{35}S]PAPS, and [^{14}C]glucose into CSA are given in Table I.

These results do not substantiate MEYER and co-workers' hypothesis that chondroitin is the immediate precursor of CSA. Chondroitin does not act as a sulphate acceptor in the system described nor does it influence the incorporation of [^{14}C]glucose into CSA. Chondroitin was found to have an inhibitory effect on the formation of CSA but since it had little effect on the transfer of sulphate from PAPS to CSA it could be affecting PAPS formation. This was shown to be the case by estimating the [^{35}S]PAPS present in the system after incubation with and without added chondroitin in the presence of $Na_2{}^{35}SO_4$. The nucleotide fraction was subjected to paper electrophoresis and the [^{35}S]PAPS measured as previously described. The concentration of [^{35}S]PAPS is much lower in the presence of chondroitin (Fig. 1). Hyaluronic acid, the isomer of chondroitin, had a similar effect.

TABLE I

EFFECT OF CHONDROITIN ON BIOSYNTHESIS OF CSA

After incubation for 2 h at 37°, the solutions were heated for 1 min at 100° and diluted with 10 ml 0.1 *M* Na_2SO_4. Carrier CSA (1 ml of 1 % solution of sodium CSA (Sigma)) was added followed by 1 ml 5 % CETAVLON[1,6]. The precipitates were allowed to stand overnight and centrifuged. Precipitates in Expts. 1 and 2 were washed 5 times with 0.1 *M* Na_2SO_4 and that in Expt. 3, 5 times with 1 % glucose. The washed precipitates were collected by filtration on paper discs, dried in a desiccator and the paper and precipitate fixed to planchets and counted with an end-window counter.

Expt.	*Tracer*	*Added chondroitin (μmoles repeating disaccharide unit)*	*Activity in CSA (counts/min)*
1*	$Na_2{}^{35}SO_4$	Nil	7,600
		1	6,100
		3	3,200
2**	[^{35}S]PAPS	Nil	103
		2	91
3***	[^{14}C]glucose	Nil	364
		2	376

* System comprised ATP, 5 μmoles; $MgCl_2$, 6 μmoles; $Na_2{}^{35}SO_4$ (carrier free), 50 μC; Tris, pH 8.2, 40 μmoles; enzyme, 0.3 ml. Total vol., 0.7 ml.

** As in Expt. 1 with the addition of cysteine hydrochloride, 3 μmoles; glucose, 5 μmoles; and 1 ml of PAPS solution containing 22,000 counts/min in place of $Na_2{}^{35}SO_4$. Total vol., 1.7 ml.

*** As in Expt. 1 with addition of glutathione, 5 μmoles and [^{14}C]glucose, 10 μC, in place of $Na_2{}^{35}SO_4$. Total vol., 0.7 ml.

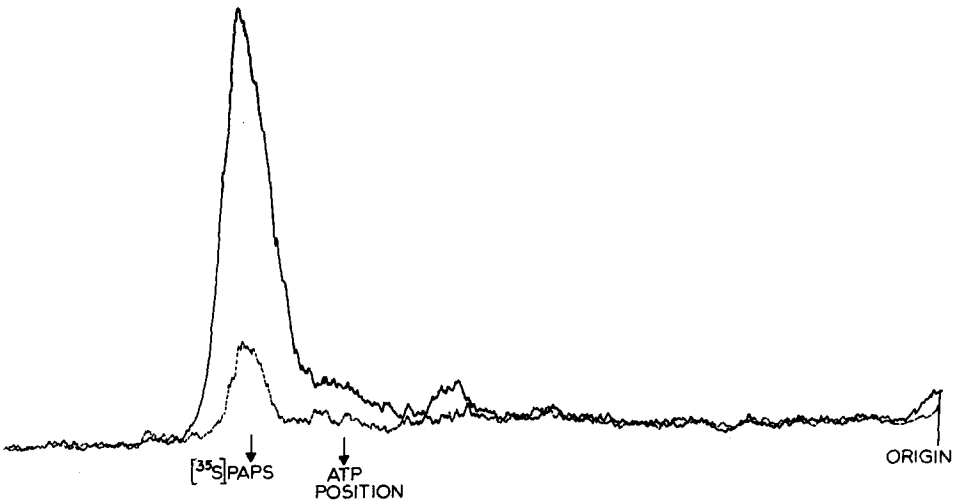

Fig. 1. Influence of chondroitin on PAPS formation. Scan of paper electrophoretogram of nucleotide fraction from incubation as in Expt. 1 of Table I. Electrophoresis was carried out in 0.025 *M* citrate buffer, pH 5.5, at 2.7 V/cm for 16 h. ——— Control; --------- Addition of 2 μmoles repeating disaccharide unit chondroitin.

These results indicate that it is likely that sulphation takes place at some stage in the biosynthesis of CSA before the formation of the final polysaccharide molecule. Further work is proceeding to try and determine at which stage sulphation occurs.

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The effect of residual peroxide on enzymic digestion of oxidized papain

In connection with studies of the amino acid sequence of papain it has been repeatedly observed that the protein after oxidation with performic acid exhibits considerable variability in its susceptibility to trypsin. In fact, one preparation of oxidized papain was found to be completely resistant to trypsin, chymotrypsin and even activated papain. This relative or complete resistance to proteolysis is now known to be caused by residual peroxide in the oxidized protein. Inasmuch as oxidation followed by