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STIMULATION OF GLYCOSAMINOGLYCAN SULFOTRANSFERASE FROM CHICK EMBRYO CARTILAGE BY BASIC PROTEINS AND POLYAMINES

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Summary

A soluble glycosaminoglycan sulfotransferase (3′-phosphoadenylylsulfate: chondroitin 4′-sulfotransferase, EC 2.8.2.5) from chick embryo cartilage has been prepared free from endogenous acceptor. The reaction with this enzyme preparation was stimulated by basic proteins and polyamines, the degree of stimulation being dependent on the chemical nature of both basic compounds and acceptor glycosaminoglycans. A maximum stimulation was obtained when protamine (basic compound) and chondroitin (acceptor) were involved in the reaction mixture at a molar ratio of protamine to repeating disaccharide units of chondroitin, 1 : 100. The stimulation of sulfotransferase activity by basic substances was much higher than that by Mn^{2+}. However, increasing the Mn^{2+} concentration immediately reduced the stimulation by basic substances. The K_m value for 3′-phosphoadenosine 5′-phosphosulfate of the sulfotransferase, when chondroitin was used as acceptor, was $1 \cdot 10^{-6}$ M in the presence of 25 μg/ml protamine, compared to $2 \cdot 10^{-5}$ M in the absence of protamine.

These observations indicate that the basic proteins and polyamines may interact with acceptor polysaccharide, thereby causing an increase in the affinity of the enzyme toward 3′-phosphoadenosine 5′-phosphosulfate.

Abbreviations: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; ΔDi-0S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galatose; ΔDi-4S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose; ΔDi-6S, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose. For chemical formula of these compounds, see Ref. 18.

Introduction

Glycosaminoglycan sulfotransferases (3′-phosphoadenylylsulfate:chondroitin 4′-sulfotransferase, EC 2.8.2.5), which catalyze sulfate transfer from PAPS to the polysaccharide chains of proteoglycan, have been shown to be located predominantly at the Golgi apparatus (e.g. in chondrocyte, see Ref. 1). Previous studies [2—6] have shown that a part of sulfotransferase activity can be solubilized by either homogenizing or sonicating cartilage or other connective tissues. Silbert et al. [7,8], on the other hand, have used the microsomes from chick embryo cartilage and demonstrated the occurrence of an efficient sulfation of endogenous proteoglycan. Studies on the mechanism of sulfation with particulate enzyme, however, have been hampered by the fact that the nature of sulfotransferases and proteoglycan acceptors can not be adequately assessed, and it seems necessary to further the enzymological studies on glycosaminoglycan sulfotransferases.

In this paper we describe the preparation of a soluble sulfotransferase fraction from chick embryo cartilage and its novel property, stimulation by basic proteins and polyamines.

Experimental

Materials

Chondroitin was prepared from the skin of squid, *Ommastrephes sloani pacificus*, according to the method of Anno et al. [9] with a slight modification in which DEAE-cellulose instead of cetylpyridinium chloride was used for purification. Crude polysaccharide, 400 mg, was applied to the DEAE-cellulose column (2.1 × 32 cm) equilibrated with 0.02 M Tris-HCl, pH 7.2, and washed with 600 ml of the same buffer. The adsorbent was eluted with 600 ml of 0.4 M NaCl in 0.02 M Tris-HCl, pH 7.2. The fractions containing uronic acid were pooled, dialyzed against distilled water, and precipitated with 2 vols. of ethanol containing 1% potassium acetate. The precipitate was collected by centrifugation, washed with ethanol, and dried in a vacuum desiccator over P_2O_5. This fraction gave a single band on cellulose acetate electrophoresis with a mobility corresponding to standard hyaluronic acid. When digested with chondroitinase-ABC and subjected to paper chromatography, 18% of the total galactosamine of this fraction remained at the origin of paper while the remainder migrated from the origin, of which more than 90% was recovered as ΔDi-0S. The material remaining at the origin has not been characterized.

Chondroitin 4/6-sulfate was prepared from epiphyseal cartilages of 14-day-old chick embryo. Epiphyseal cartilages obtained from 50 embryos were digested with pronase (6 mg/ml) in 5 vols. of 0.05 M Tris-HCl, pH 8.0 at 37°C overnight. A 5% concentration of trichloroacetic acid was made and the solution was centrifuged. The supernatant fluid was dialyzed against water and mixed with 2 vols. ethanol containing 1% potassium acetate. The precipitate was collected by centrifugation, washed with ethanol and vacuum-dried. The yield was 50 mg. This fraction gave a broad band between the dermatan sulfate zone and the chondroitin sulfate zone on cellulose acetate electrophoresis. When digested with chondroitinase-ABC, it yielded ΔDi-4S, ΔDi-6S and ΔDi-0S in the ratio of 27 : 54 : 19.

A 4-sulfate-rich chondroitin sulfate (which will be referred to as 'chondroitin 4-sulfate') was prepared from bovine nasal cartilage using the same method as described above, and further purified by DEAE-Sephadex A-50 chromatography according to Schmidt [10] with some modification, in which a molarity gradient (0.5—1.2) of NaCl in 0.1 M pyridine/acetic acid buffer (pH 3.5) was used for the elution. After digestion with chondroitinase-ABC, it yielded ΔDi-4S, ΔDi-6S and ΔDi-0S in the ratio of 81 : 14 : 5.

The following materials were prepared by previously described methods: Chondroitinase-ABC from *Proteus vulgaris* NCTC 4636, ΔDi-4S, ΔDi-6S, and ΔDi-0S from chondroitin sulfate [11]; ^{35}S-labeled PAPS with specific activity $9 \cdot 10^9$ cpm/μmol and unlabeled PAPS [12]; protamine chloride from protamine sulfate by passing through a column of Dowex 1 (Cl^-) [3].

The following commercial materials were used: DEAE-cellulose from Brown Co., Keene, NH.; DEAE-Sephadex A-50 from Pharmacia, Uppsala; egg white lysozyme (hydrochloride form) from Boehringer Mannheim Yamanouchi, Tokyo; protamine sulfate (salmon, grade I), and calf thymus histone (Type II) from Sigma, St. Louis; spermine tetrahydrochloride and spermidine trihydrochloride from Nakarai Chemicals, Co., Kyoto. All other chemicals were reagent grade.

Generous gifts of the following materials are acknowledged: chondroitin 6-sulfate from human meniscus (fraction 40 in Ref. 13) from Dr. H. Habuchi, Nagoya University; and Pronase-P from Kaken Kagaku Co., Tokyo.

Enzyme assay

Assay of sulfotransferase was performed essentially as described previously [4]. The incubation mixtures contained, in a final volume of 100 μl: 5 μmol Tris-HCl, pH 7.5, 0.2 μmol $MnCl_2$, 1.0 μmol reduced glutathione, 0.05 nmol (about $5 \cdot 10^5$ cpm) ^{35}S-labeled PAPS, and the enzyme, acceptor and basic protein or polyamine as indicated in the individual experiment.

After incubation at 37°C for 20 min, the reactions were stopped by immersing the reaction tubes in a boiling water bath for 1 min. Insoluble materials, if present, were digested with pronase (0.1 mg/reaction mixture) for 1 h at 37°C. The volume of the reaction mixtures was adjusted to 300 μl and 700 μl of 95% ethanol/1% potassium acetate/0.5 mM EDTA were added to precipitate glycosaminoglycans. After 30 min in an ice bath, the precipitates formed were collected by centrifugation at 10 000 × *g* for 10 min. The precipitates were dissolved in 300 μl water and the precipitation with ethanol was repeated three times. The final precipitates were dissolved in 100 μl water and a 20 μl portion of the solution was counted by liquid scintillation. For determining the transfer of sulfate to position 4 and position 6 of the acetylgalactosamine moiety, the remainder of each polysaccharide fraction was digested with chondroitinase-ABC [16] and disaccharides (ΔDi-4S and ΔDi-6S) formed were separated by paper chromatography with the solvent system described below. The regions corresponding to ΔDi-4S and ΔDi-6S were cut out and their radioactivities were measured.

Other methods

Radioactivity was determined in a Packard Tri-Carb liquid scintillation

spectrometer with the solvent system recommended by the manufacturer. Glucuronic acid was determined by the method of Dische [14] with glucuronolactone as standard. Protein was determined by the method of Lowry et al. [15] with bovine serum albumin as standard.

Paper chromatography was performed in a descending technique with the solvent system, 1-butanol/acetic acid/1 M ammonia (2 : 3 : 1, v/v) [16] at room temperature. Toyo No. 51A paper was used.

Electrophoresis of glycosaminoglycans was carried out on 6-cm long strips of Separax (cellulose acetate film) at a constant current of about 1 mA/cm for 20 min. The buffer used was pyridine/acetic acid/water (1 : 9 : 115, v/v), pH 3.5, and the strips were stained according to the method of Seno et al. [17].

Results

Preparation of a soluble sulfotransferase fraction from chick embryo cartilage

Epiphyseal cartilage of tibias and femurs of 13-day-old chick embryos were homogenized with 5 vols. ice-cold 0.02 M Tris-HCl, pH 7.2, containing 0.01 M 2-mercaptoethanol and 10% glycerol (buffer 1). The homogenate was centrifuged at 10 000 $\times g$ for 10 min and the supernatant fluid was collected and dialyzed overnight against 100 vols. of buffer 1. The crude extract (19 mg as protein) was applied to a DEAE-cellulose column (2.1 $\times$ 15 cm) equilibrated with buffer 1, and washed with 200 ml of the same buffer. The column was developed by linear gradient elution with 250 ml buffer 1 in the mixing flask and 250 ml 0.6 M NaCl in the same buffer in the reservoir. The flow rate was 60 ml/h and fractions of 8 ml were collected (Fig. 1). The fractions containing the major portion of the enzyme activity (indicated by the horizontal bar in Fig. 1) were pooled, concentrated to 6.4 ml in an ultrafiltration apparatus, using Diaflo PM 10 membrane, and dialyzed against 1 l buffer 1. The soluble sulfotransferase preparation thus obtained had no uronic acid-containing material and, therefore, showed no significant enzyme activity unless a suitable exogenous acceptor was added to the reaction mixtures. A significant PAPS-degrading activity was still found in the preparation, but, under the standard assay condition described under 'Enzyme assay', the incorporation of sulfate into glycosaminoglycan proceeded linearly as the quantity of enzyme increased up to 20 μg protein; this fraction was used for the experiments described below.

Effects of acceptor glycosaminoglycan and basic substances on the soluble sulfotransferase

Effects of protamine on the sulfotransferase activities toward various acceptor glycosaminoglycans are shown in Fig. 2. In these experiments, a constant amount (50 nmol as glucuronic acid) of each acceptor polysaccharide and varying amounts of protamine were involved in the reaction mixtures. The degree of stimulation by protamine depended on the structure of acceptor polysaccharides. In the absence of protamine, chick embryo chondroitin 4/6-sulfate was the most efficient acceptor, whereas in the presence of protamine a marked stimulation was detected in the reaction with chondroitin. The sulfotransferase activity on chondroitin 4/6-sulfate was also stimulated by protamine but this

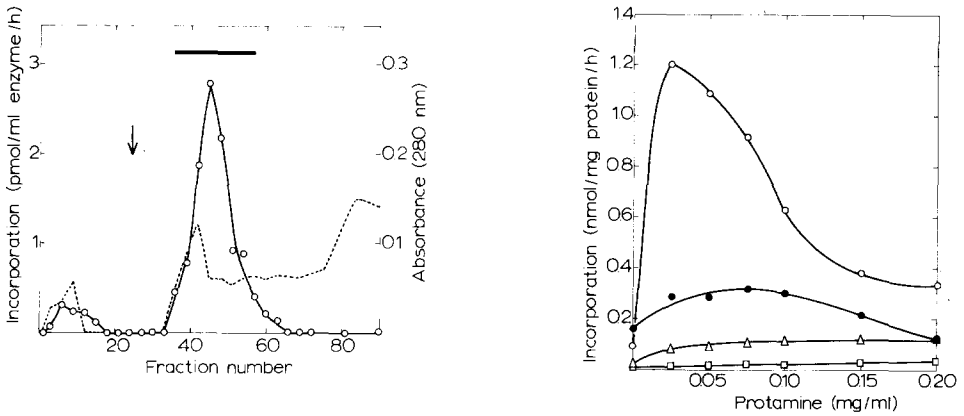

Fig. 1. DEAE-cellulose chromatography of chick cartilage sulfotransferase. Fractions were obtained as described in the text and checked for sulfotransferase activity (○———○) and ultraviolet (280 nm)-absorbing material (- - - - -). The arrow indicates the start of gradient. The enzyme assay was carried out as described under 'Enzyme assay'. For the assay, chick cartilage chondroitin 4/6-sulfate (50 nmol as glucuronic acid) was added as acceptor. The incorporation of sulfate into polysaccharide fraction was measured.

Fig. 2. Effects of protamine on the incorporation of sulfate into various acceptors. The assay was carried out as described under 'Enzyme assay'. Chondroitin (○———○), chick cartilage chondroitin 4/6-sulfate (●———●), chondroitin 4-sulfate (△———△), or chondroitin 6-sulfate (□———□) was used as acceptor at the concentration of 50 nmol (as glucuronic acid) per 100 μl reaction mixture, with varying amounts of protamine chloride and the soluble enzyme preparation (9.4 μg protein). The incorporation of sulfate into polysaccharide fraction was measured.

effect appears to be due to the presence of 20% non-sulfated component in the preparation (see 'Materials'), since analysis with chondroitinase of the sulfated products indicated that about 13% and 85% of the total radioactivity incorporated, exist in the product molecule as acetylgalactosamine 4-sulfate and acetylgalactosamine 6-sulfate units, respectively. Though both chondroitin 4-sulfate and chondroitin 6-sulfate served as relatively poor acceptors, a significant stimulation by protamine was observed.

Using chondroitin as acceptor, the effects of various basic substances were investigated (Table I). At each concentration given in Table I, the stimulation by protamine, spermine, or spermidine showed a maximum, whereas the stimulation by histone reached a plateau, and that by lysozyme continued to increase up to 4 mg/ml. The incorporation into position 6 of the acetylgalactosamine moiety was stimulated more greatly than incorporation into position 4 by any of the basic substances. The incorporation of sulfate into the material remaining at the paper origin after chondroitinase-ABC digestion was also stimulated, but no further investigation about this fraction has been carried out. Except for those fractions shown in Table I, only slight incorporation (less than 2% of total incorporation) was found in a fraction which migrated near ΔDi-0S on paper chromatogram and was presumably assigned to free acetylgalactosamine 4-sulfate [18].

Variation of optimal protamine concentration with chondroitin concentration

If some interaction between the basic substance and acceptor glycosamino-

TABLE I

CHONDROITINASE-ABC DIGESTION OF PRODUCTS FORMED BY SULFOTRANSFERASE REACTION IN THE PRESENCE OF BASIC SUBSTANCES

Assay conditions are described under 'Enzyme assay'. Chondroitin (50 nmol as glucuronic acid) was used as acceptor with the indicated amounts of basic substances and the soluble enzyme preparation (9.4 μg protein). The labeled products were digested with chondroitinase-ABC and subjected to paper chromatography. The incorporation of sulfate into ΔDi-4S, ΔDi-6S, and resistant polymers (materials remaining at the paper origin) was measured.

Basic substances	Incorporation of sulfate (nmol/mg protein per h)		
	ΔDi-4S	ΔDi-6S	Resistant
None	0.014	0.063	0.006
Protamine (0.025 mg/ml)	0.071	1.08	0.051
Histone (0.1 mg/ml)	0.091	0.98	0.032
Lysozyme (4 mg/ml)	0.029	0.23	0.010
Spermine (1 mM)	0.029	0.27	0.012
Spermidine (4 mM)	0.021	0.17	0.009

glycan occurs in the reaction mixture, the optimal concentration of each basic substance shown in Table I may be influenced by acceptor concentration. In Fig. 3, effects of the concentration of chondroitin on the amount of protamine required for maximum stimulation are shown. The optimal concentration of protamine was influenced by acceptor concentration. When the amount of chondroitin (as glucuronic acid) was varied from 25 nmol (Fig. 3A) to 50 nmol (Fig. 3B) and to 100 nmol (Fig. 3C), the concentration of protamine for maximum stimulation increased from 1.2 μg, to 2.0 μg and 5.0 μg, respectively. If the optimal concentration of protamine is expressed relative to mol

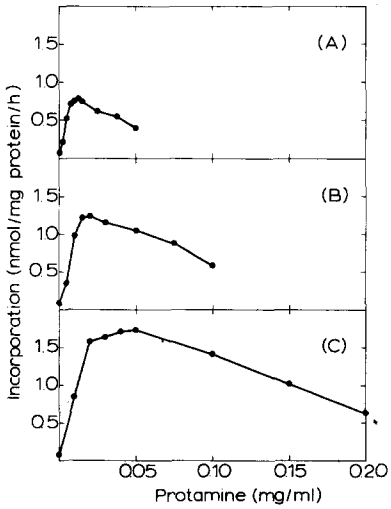

Fig. 3. Effects of acceptor concentration on the stimulation of sulfate incorporation. The assay was carried out as described under 'Enzyme assay'. Chondroitin (25 nmol, 50 nmol and 100 nmol as glucuronic acid in A, B and C, respectively) was used as acceptor, with varying amounts of protamine and the soluble enzyme preparation (9.4 μg protein). The incorporation of sulfate into polysaccharide fraction was measured.

glucuronic acid residue of chondroitin, it becomes a constant within a range of 40—50 μg/μmol glucuronic acid, regardless of the amounts of chondroitin added to the reaction mixtures. Since the molecular weight of protamine is about 4000 [19], the molar ratio of protamine to repeating unit of acceptor polysaccharides to give a maximum stimulation is approx. 1 : 100.

In contrast to the acceptor substrate, the donor substrate, PAPS, caused no change of the optimal protamine concentration (data not shown). Furthermore, increase of enzyme concentration from 4.7 μg (as protein) to 9.4 μg, in the presence of 50 nmol of added chondroitin, had little effect on the amount of protamine required for a maximum stimulation (20 μg/ml), suggesting that protamine has no effect on the enzyme(s) itself.

These observations, taken together, suggest that the stimulation of sulfotransferase by protamine is due not to a direct interaction between enzyme molecule and protamine but to a binding of protamine to acceptor polysaccharide at a suitable molar ratio. The acceptor protamine complex may be a more favorable substrate for sulfotransferase than the acceptor itself.

Effects of Mn^{2+} concentration on incorporation of sulfate

It is well known that polyamines activate various enzymes [20], and different relations between divalent metal ions and polyamines have been reported. In some cases [21—23], polyamines can substitute for Mg^{2+} required in enzyme reactions, and it is postulated that polyamines bind to substrate, tRNA, inducing configurational changes favourable for the enzyme reactions. In some other cases [24,25], the apparent K_m values for divalent metal ions were lowered on adding polyamines. To see what relationship between Mn^{2+} and polycationic substances may exist in the sulfotransferase system, the effects of Mn^{2+} on the sulfate incorporation into chondroitin were examined in the presence or absence of basic substances, as shown in Fig. 4. In the absence of basic substances, addition of $MnCl_2$ at 0.5—10 mM resulted in a limited stimulation of sulfotransferase activity. The stimulation caused by the presence of protamine (25 μg/ml) or spermine (1 mM) was much higher than the stimulation by Mn^{2+} alone. However, as Mn^{2+} concentration was increased in the presence of the constant concentration of protamine or spermine, the rate of sulfation decreased immediately. It is also likely that protamine or spermine can replace Mn^{2+}, required for sulfotransferase reaction, since the addition of 1 mM EDTA (Fig. 4, triangles) to reaction mixtures without manganese salt caused little inhibition of sulfate incorporation if protamine or spermine were present.

Effects of PAPS concentration on the incorporation of sulfate

Effects of PAPS concentration on the rate of sulfate transfer to chondroitin, in the presence or absence of protamine, are shown in Fig. 5. Activation of sulfotransferase by protamine was marked at lower concentrations of PAPS. The apparent K_m value for PAPS determined by a Lineweaver-Burk plot was $2 \cdot 10^{-5}$ M in the absence of protamine and $1 \cdot 10^{-6}$ M in the presence of protamine. On the other hand, the relative value of V in the presence of protamine was only 1.3-times as large as that in the absence of protamine. Since, however, the enzyme preparation is apparently a mixture of two sulfotransferase species (e.g. 4-sulfotransferase and 6-sulfotransferase), the attempted estimation of K_m and V values for the present system is not valid.

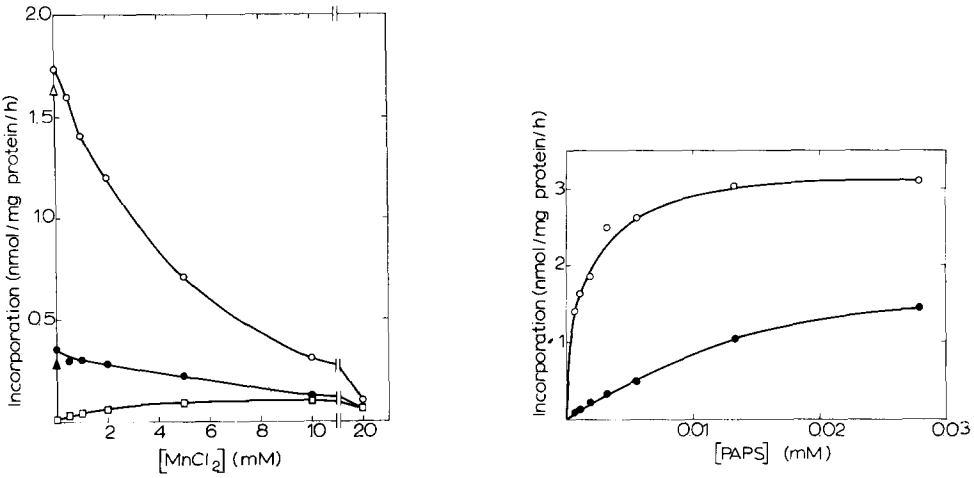

Fig. 4. Effects of Mn^{2+} concentration on the incorporation of sulfate. The assay was carried out as described under 'Enzyme assay' except that Mn^{2+} concentration was varied as indicated. Chondroitin (50 nmol as glucuronic acid) as acceptor and the soluble enzyme preparation (9.4 μg protein) were added in the absence of basic substances (□———□), or in the presence of 25 μg/ml protamine chloride (○———○) or 1 mM spermine (●———●). EDTA was added at 1 mM to the reaction mixture involving protamine (△) or spermine (▲) without added manganese salt. The incorporation of sulfate into polysaccharide fraction was measured.

Fig. 5. Effects of the concentration of PAPS on the incorporation of sulfate. The assay was carried out as described under 'Enzyme assay' except that the PAPS concentration was varied, as indicated by adding unlabeled PAPS. Chondroitin (50 nmol as glucuronic acid) as acceptor and the soluble enzyme preparation (9.4 μg protein) were added in the absence of basic substances (●———●), or in the presence of 25 μg/ml protamine (○———○). The incorporation of sulfate into polysaccharide fraction was measured.

Discussion

The results reported here demonstrate the stimulation of glycosaminoglycan sulfation by basic proteins or polyamines. From the observations described above, it seems likely that the basic substances may bind to acceptor glycosaminoglycans so as to change the configuration of glycosaminoglycans to a more susceptible form. A similar observation has been made in the study of sperm hyaluronidase [26].

Since the stimulation of sulfotransferase by protamine was revealed at relatively low concentrations of PAPS, it would have been difficult to detect any stimulatory effects of protamine in experiments where a saturated concentration of PAPS were used for the determination of sulfotransferase activity. Although the biological significance of the enzyme activation by basic compounds is unknown, it is tempting to speculate that in vivo some basic compounds may play a role in the regulation of sulfation of proteoglycans under conditions where intracellular PAPS concentration is less than the K_m value of sulfotransferase.

Since the sulfotransferase preparation used still contains some PAPS-degrading activity, a possibility must be considered that the apparent stimulation of sulfate incorporation is secondary to inhibition of the PAPS-degrading enzyme.

This possibility is unlikely since the rate of degradation of PAPS did not change in the presence of protamine: the amount of $^{35}SO_4^{2-}$ released from ^{35}S-labeled PAPS/min per mg enzyme protein was $4.6 \cdot 10^5$ cpm in the presence of 2.5 μg protamine, compared to $4.0 \cdot 10^5$ cpm in the absence of protamine.

Activation of the sulfate transfer to position 6 of the acetylgalactosamine moiety was always higher than that to position 4. These results can be taken as a reflection of the fact that different enzymes are responsible for the sulfation of position 6 and position 4 of acetylgalactosamine moiety, the enzyme catalyzing the sulfation of position 6 being more sensitive to the basic substances.

The occurrence of lysozyme in various cartilages has been widely described [27—30]. It seems unlikely, however, the lysozyme does influence sulfotransferase activity in the tissues, since the stimulatory effect of lysozyme was detected only at very high lysozyme concentrations and most lysozyme is believed to occur in the extracellular matrix [27]. Another basic protein, which inhibits collagenolytic activity has been reported in bovine cartilage [31] but, at the moment, it is not known whether this protein may affect sulfotransferase activities. Further work is necessary to elucidate the physiological significance of the stimulation of sulfotransferase by basic substances.

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References

1 Kimata, K., Okayama, M. and Suzuki, S. (1971) Biochim. Biophys. Acta 237, 606—610
2 Robinson, H.C. (1969) Biochem. J. 113, 543—546
3 Meezan, E. and Davidson, E. (1967) J. Biol. Chem. 242, 1685—1689
4 Habuchi, O., Yamagata, T. and Suzuki, S. (1971) J. Biol. Chem. 246, 7357—7365
5 Suzuki, S. and Strominger, J.L. (1960) J. Biol. Chem. 235, 257—266
6 Kimata, K., Okayama, M., Oohira, A. and Suzuki, S. (1973) Mol. Cell. Biochem. 1, 211—228
7 Silbert, J.E. and DeLuca, L. (1969) J. Biol. Chem. 244, 876—881
8 DeLuca, S., Richmond, M.E. and Silbert, J.E. (1973) Biochemistry 12, 3911—3915
9 Anno, K., Kawai, Y. and Seno, N. (1964) Biochim. Biophys. Acta 83, 348—349
10 Schmidt, M. (1962) Biochim. Biphys. Acta 63, 346—348
11 Yamagata, T., Saito, H., Habuchi, O. and Suzuki, S. (1968) J. Biol. Chem. 243, 1523—1535
12 Robbins, P.W. (1962) Methods Enzymol. 5, 964—977
13 Habuchi, H., Yamagata, T., Iwata, H. and Suzuki, S. (1973) J. Biol. Chem. 248, 6019—6028
14 Dische, Z. (1947) J. Biol. Chem. 167, 189—198
15 Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
16 Saito, H., Yamagata, T. and Suzuki, S. (1968) J. Biol. Chem. 243, 1536—1542
17 Seno, N., Anno, K., Kondo, K., Nagase, S. and Saito, S. (1970) Anal. Biochem. 37, 197—201
18 Suzuki, S. (1960) J. Biol. Chem. 235, 3580—3588
19 Ando, T. and Watanabe, S. (1969) Int. J. Protein Res. 1, 221—224
20 Tabor, C.W. and Tabor, H. (1976) Ann. Rev. Biochem. 45, 285—306
21 Leboy, P.S. (1970) Biochemistry 9, 1577—1584
22 Young, D.V. and Srinivasan, P.R. (1971) Biochim. Biophys. Acta 238, 447—463
23 Takeda, Y., Ohnishi, T. and Ogiso, Y. (1976) J. Biochem. 80, 463—469
24 Baker, A.P. and Hillegass, L.M. (1974) Arch. Biochem. Biophys. 165, 597—603
25 Yoshida, S., Masaki, S. and Ando, T. (1976) J. Biochem. 79, 895—901

26 Doak, G.A. and Zahler, W.L. (1979) Biochim. Biophys. Acta 570, 303—310
27 Kuettner, K.E., Eisenstein, R., Soble, L.W. and Arsenis, C. (1971) J. Cell Biol. 49, 450—458
28 Guenther, H.L., Sorgente, N., Guenther, H.E., Eisenstein, R. and Kuettner, K.E. (1974) Biochim. Biophys. Acta 372, 321—334
29 Schmidt, A., Rodegerdts, U. and Buddecke, E. (1978) Calcif. Tissue Res. 26, 163—172
30 Greenwald, R.A., Josephson, A.S., Diamond, H.S. and Tsang, A. (1972) J. Clin. Inv. 51, 2264—2270
31 Kuettner, K.E., Hiti, J., Eisenstein, R. and Harper, E. (1976) Biochem. Biophys. Res. Commun. 72, 40—46