Enzymic transsulfation from a phenol to carbohydrates

According to our study on the incorporation of ^{35}S-sulfate into different organs of *Charonia lampas* (a marine gastropod), radioactive sulfur was located in the mucous gland 69 h after the injection of ^{35}S-sulfate[1], and the major part of the fixed ^{35}S was found[2] in the sulfate group of charoninsulfuric acid, a glucan polysulfate present in the gland[3]. However, the incorporation did not occur with an acetone powder of the gland.

We now report that the sulfation of charoninsulfuric acid was accomplished in the presence of acetone-dried powder when *p*-nitrophenylsulfuric acid was present as a sulfate donor in an appropriate incubation mixture. No sulfation took place with a heat-denatured preparation.

One gram of acetone powder, prepared from the mucous gland in the usual manner, was suspended in 10 ml 0.15 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer, pH 7.4, containing 0.2 g KCl and 0.01 ml chloroform. The suspension was incubated at 35° C for 20 h. During the incubation, a part of charonin sulfate in the suspension was digested and glucose, maltose and three unidentified oligosaccharides were produced. 40 μmoles potassium *p*-nitrophenyl ^{35}S-sulfate, containing about 20 microcuries of the radioisotope per mg, were then added to the suspension, which was reincubated for another 20 h. Under these conditions, 10 μmoles *p*-nitrophenol were liberated. The reaction was stopped by boiling for 3 min, and 0.03 ml of the mixture was put on a filter paper strip (3 × 40 cm) as a thin band. After the strip was dried, a mixture of *n*-butanol, ethanol, water and ammonia (40:12:20:1) was allowed to ascend in the manner usual in paper chromatography until it was 5 cm from the top.

The autoradiographic tracings are shown in Fig. 1. Radioactive substances appeared at the origin and somewhat above the inorganic sulfate level, while the zero-time sample did not contain any of these substances. Experiments without *p*-nitrophenyl ^{35}S-sulfate also showed no evidence for the formation of these substances even in the presence of corresponding amounts of ^{35}S-K_2SO_4. The fact that the origin was easily seen as a reddish band on a blue background by the metachromatic stain with 0.125% toluidine blue solution suggested that the radioactive charoninsulfuric acid was located at this point. This was confirmed by extracting the charoninsulfuric acids from each 10 ml of the reaction mixture as described in the preceding paper[2], and then hydrolysing with 6 *N* HCl for 4 h in a boiling water bath. The resulting sulfate was precipitated as the benzidine salt for the determination of the radioactivity. It was shown that 46 c.p.m./cm²

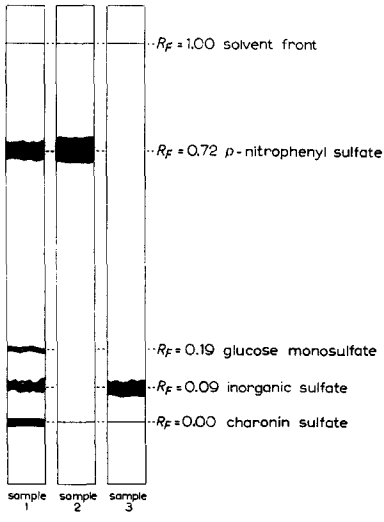


Fig. 1. Detection of ^{35}S-containing products by paper autoradiography. Sample 1: complete system, incubated for 20 h at 35°, contained 40 μmoles *p*-nitrophenyl ^{35}S-sulfate in 10 ml suspension of acetone powder. Sample 2: complete system in which reaction was stopped by boiling at zero time. Sample 3: system without *p*-nitrophenyl ^{35}S-sulfate contained 40 μmoles ^{35}S-K_2SO_4. Solvent: *n*-butanol/ethanol/water/ammonia (40/12/20/1).

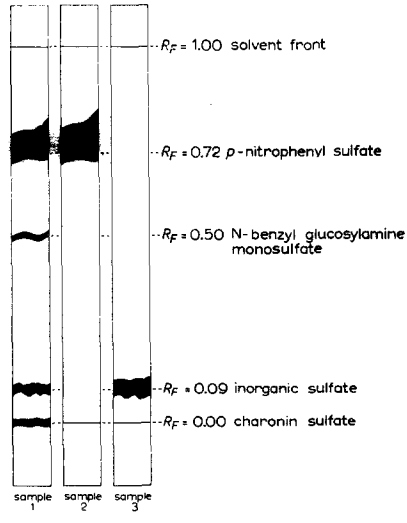


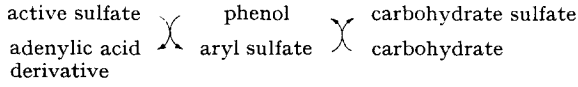
Fig. 2. Paper autoradiograph of samples treated with benzylamine. Identical samples in Fig. 1 were previously treated with benzylamine and then run in the same solvent as for Fig. 1.

(measured at infinite thickness) of the radioactive sulfur was transferred from *p*-nitrophenyl sulfate to charonin sulfate, while no such reaction was observed at zero time nor when the *p*-nitrophenyl ^{35}S-sulfate in the system was replaced by ^{35}S-K_2SO_4.

The following considerations indicate that the other radioactive substance with an R_F value of 0.19 is glucose monosulfate: (1) 1.5 mg synthetic glucose-6-monosulfate was dissolved in 0.03 ml of the test solution and chromatographed on paper as described above. Glucose monosulfate appeared as a brown band on heating at 105° C for 5 min after spraying with an aniline hydrogen phthalate reagent. It was observed that the autoradiographic tracing overlapped with this visualisation. (2) If the substance is a reducing glucose monosulfate, it must be converted by benzylamine into the corresponding N-benzyl glycosylamine, and thus a considerable increase in R_F value may be effected as demonstrated by BAYLY AND BOURNE[4]. After 0.03 ml of the test solution was put on the paper, 0.035 ml of a 4% solution of benzylamine in methanol was superposed. The paper was heated at 85° C for 5 min, and then the same solvent as described above was allowed to ascend. The autoradiograph thus obtained is shown in Fig. 2. As can be seen, a considerable increase in R_F value (about 2.5 fold) was obtained.

From these experiments, it was concluded that ^{35}S-sulfate was incorporated into carbohydrates from *p*-nitrophenyl ^{35}S-sulfate by enzymic transsulfation and no incorporation took place from inorganic ^{35}S-sulfate. Further work is now in progress on the purification of the enzyme participating in the transsulfation and on the isolation of natural aryl sulfates in the mucous gland. However, it should be noted here that the transsulfation was inhibited by phosphate and fluoride in the same way as the aryl sulfate hydrolysis by arylsulfatases. This seems to suggest the participation of arylsulfatase itself in the reaction.

In the studies on the conjugation of sulfate with phenols, it was demonstrated by several workers that the primary step of the conjugation is an enzymic activation of inorganic sulfate by ATP. Recently HILZ AND LIPMANN[5] and DE MEIO AND WIZERKANIUK[6] have found that the active sulfate is an adenylic acid derivative. Their finding and the present evidence invite a tentative formulation of the sulfate transfer:



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Factors altering the activities of ribonuclease A and ribonuclease B

The specific question which led to the present investigations was that of the relation between the specificity of the major ribonuclease components (ribonuclease A and ribonuclease B) in the breakdown of ribonucleic acid (RNA).

In earlier investigations[1,2], we showed that ribonuclease B liberated 9.8 to 13.8% of guanylic acid, while crystalline ribonuclease, which contained both components A and B, liberated only 0.9 to 1.2%. Furthermore some synthetic activities of crystalline ribonuclease observed by HEPPEL, WHITFELD AND MARKHAM[3,4], were demonstrated[2] to occur with ribonuclease B.

The action of crystalline ribonuclease appears complex in nature, and depends on the proportion of the 2 fractions, A and B, acting together. The action of ribonuclease A and ribonuclease B, alone and combined in various proportions, has been investigated. The results reported here