Oxidation by Partially Hydrolyzed Polysaccharides

KALE JUVA and EINO KULONEN

Department of Medical Chemistry, University of Turku, Finland

When certain polysaccharides are hydrolyzed partially with hydrochloric acid in sealed tubes compounds are formed which catalyze the oxidation of many aromatic substances (*e.g.* phenol, *p*-phenylenediamine), especially in the presence of hydrogen peroxide [1]. The chemical nature of the catalysts is unknown, but they are acidic and contain a carbonylic group [2]. Hydrogen peroxide degrades hyaluronic acid [3] in the presence of ascorbic acid but no catalyst is formed after this "oxidative depolymerization" (see below). The oxidizing ability may contribute in the production of humins and other artefacts in hydrolyzates. For example, an increased oxidation of methionine in acid hydrolysis in the presence of carbohydrates has been described [4].

For *testing* equal volumes of the solutions in question, 1.5 % hydrogen peroxide and ammoniacal water-saturated phenol or fresh aqueous 1 % *p*-phenylenediamine (Merck) were mixed in test tubes. Controls were run with water instead of hydrolyzate. The results were recorded after 1 h at room temperature, either visually or with a spectrophotometer at 500 mμ.

The *partial hydrolyzate* was made with 2 N HCl (0.33 % of polysaccharide) keeping the sealed tubes overnight at 100°C. The acid was removed by repeated evaporations on the water bath. The catalyzing agent was dialysable. The catalytic activity is dependent on the degree of polymerization.

Ascorbic acid did not influence the reaction in this system, even when added 2 h before the *p*-phenylenediamine.

The *hyaluronate* complex used in the present investigation was prepared from umbilical cords by 1 % NaCl extraction, precipitation with "Cetavlon", solution in 1.25 M sodium chloride and fractional precipitation by lowering the salt concentration below 0.3 M. Cetavlon was removed with Fuller's earth and the solution lyophilized [5]. Before the lyophilization the fluid contained dry matter 2.6 mg/ml, uronic acid (Dische) 1.07 mg/ml, glucosamine (Blix) 0.45 mg/ml, and protein 0.62 mg/ml.

The *depolymerization with ascorbic acid* was performed in 0.2 % solution of hyaluronate, buffered to pH 6.8 with 0.0067 M phosphate and containing 0.8 % NaCl. After addition of 0.020 % H_2O_2 no change occurred, but after further addition of 0.050 % ascorbic acid the relative viscosity fell in 20 min to a tenth ($\eta_{rel} = 1.5$). The fluid was dialyzed against tap water and lyophilized. The partial hydrolyzate from this degraded preparation had no catalytic activity.

Purification of the catalyst was carried out from 82.2 mg of HCl-treated material using a cellulose powder (Whatman) column (28 × 2 cm) and butanol (almost saturated with water):acetic acid (19:1) as the solvent [6]. Five ml fractions were collected and screened with spot tests. Fractions No. 20—30 and 170—185 were positive with aniline phthalate, but with ammoniacal phenol brown spots were obtained

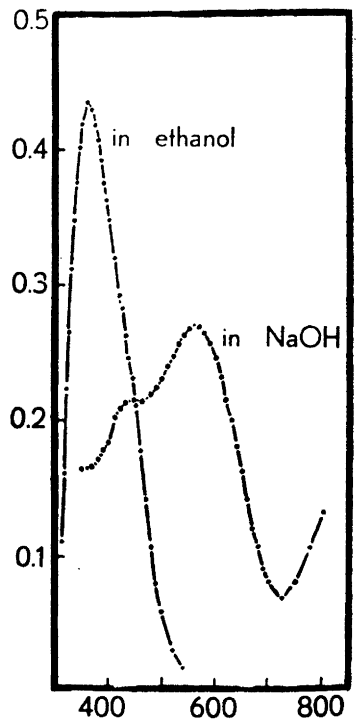

Fig. 1. The absorption spectra of the 2,4-dinitrophenylderivative of the isolated preparation. Abscissa: wavelength in mμ, ordinate: extinction.

only from fractions 30—50. They were combined and evaporated.

The residue weighed 5.6 mg and was hydrolyzed in 0.56 ml N sulfuric acid for 5 h at 100°C. The digest was neutralized with $Ba(OH)_2$ to pH 5.8. The supernatant was evaporated, the residue dissolved in 0.05 ml of water and chromatographed with butanol:pyridine:water (5:3:2). No distinct carbohydrate spots were obtained with aniline phthalate, but with ninhydrin several spots, one coinciding with that of glucosamine. The test with aniline for furfurol [7] was negative. 2,4-Dinitrophenylhydrazine (dissolved into 2 N H_2SO_4) gave a precipitate, which was collected and submitted to spectroscopic examination (Fig. 1). The sharp change to violet in alkali seems worthy of mention.

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Studies on Ester Sulfates

1. Application of Two-Dimensional Paper Chromatography in Studies on the Biosynthesis of Ester Sulfates

ANDERS VESTERMARK and HARRY BOSTRÖM

Department of Metabolic Research, the Wenner-Gren Institute of Experimental Biology, Stockholm, Sweden

In several recent biochemical studies on ester sulfate compounds, great advantage has been taken of the selective incorporation of ^{35}S-sulfate in these compounds. Mainly on the grounds of their high sensitivity, different isotope methods, such as autoradiography of electropherograms on body fluids and tissue extracts, have been used successfully in this field.

A somewhat more detailed picture of the sulfatation of different compounds in various *in vitro* systems could be expected to result from the use of two-dimensional paper chromatography combined with autoradiography. For this reason, a screening study along these lines was undertaken. Some observations made during this work will be briefly reported in the following.

In one type of experiment, 2-mg slices of liver from albino rats were incubated in small test tubes in 100 µl of Krebs-Ringer bicarbonate solution and 10 µl of a solution of ^{35}S-labeled sulfate containing 10 mc/ml. A gas mixture consisting of 93.5 % of oxygen and 6.5 % of carbon dioxide was insufflated into each test tube during incubation.

In another type of experiment, 40 µl of a cell-free supernatant of liver homogenate prepared according to Roy [1] was mixed with 50 µl of a buffer solution, containing equal parts of 0.3 M KH_2PO_4/K_2HPO_4 buffer, pH 6.8, 0.03 M K_2SO_4 and 0.005 M $MgCl_2$. This mixture was combined with 10 µl of 0.04 M ATP and 10 µl of the ^{35}S-labeled sodium sulfate solution.

When these types of sulfatation system were used for studies on sulfatation of different substances, 10 µl of a 10 mM solution of the substance in alcohol or water was added to an empty test tube. The solvent was evaporated *in vacuo*. The slices or supernatant systems were added to this tube.

In each series of experiments, one tube with 10 µl of water and another with 10 µl of alcohol, to which the relevant sulfatation system was added, were run as controls.

In all experiments, 5 µl-samples of the medium were taken 45 and 120 min after starting incubation, for two-dimensional ascending chromatography according to Datta, Dent and Harris [2]. Solvent I: Phenol-water (400 g + 100 g). A beaker containing concentrated H_3N was placed in the chromatographic tank. Solvent II: Butanol — 2 N H_3N (250 ml + 250 ml).

After drying the papers in a stream of hot air from an electric hair-drying fan, the chromatograms were subjected to autoradiography on Gevaert Curix X-ray film, exposed for one week in a wooden screw press and then developed with Gevaert G 150.