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SOLVOLYTIC DESULFATION OF 2-DEOXY-2-SULFOAMINO-D-GLUCOSE AND D-GLUCOSE 6-SULFATE

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ABSTRACT

Solvolytic desulfation of the pyridinium salts of 2-deoxy-2-sulfoamino-D-glucose and D-glucose 6-sulfate in dimethyl sulfoxide containing 5% of water or methanol was studied to develop a method for selective *N*-desulfation of heparin. The first-named salt was the most susceptible to *N*-desulfation.

INTRODUCTION

The loss of anticoagulant activity of heparin when some of the *N*-sulfate groups are liberated indicates the close relationship of these groups with biological activity[1–3]. We recently suggested an explanation for the extreme lability of these groups to acid hydrolysis[4]. In order to elucidate the fine structure of heparin and to clarify the relationship between chemical structure and biological activity, it was essential to eliminate the *N*-sulfate groups without hydrolyzing the glycosidic and *O*-sulfate bonds.

To date, *N*-desulfation of heparin is obtained[2,5] by hydrolysis with 0.04M hydrochloric acid at 100°, and by autohydrolysis of heparinic acid obtained by the treatment of heparin with a cation-exchange resin[6,7], but both methods lead to liberation of *O*-sulfate groups[6–8].

Benkovic and Dunikoski[9] reported that in the hydrolysis of 2-[4(5)-imidazolyl]-phenyl sulfate and 4(5)-(2′-hydroxyphenyl)imidazole *N*-sulfate in 50% 1,4-dioxan–water at pH 4-7 the release of *N*-sulfate groups was 70 times faster than that of *O*-sulfate groups, and Candlin and Wilkins[10] and Fleischfresser and Lauder[11] concluded that the unimolecular decomposition of the zwitterion form of sulfamic acid was the rate-determining step. Nagasawa and Yoshidome[12] described the rapid decomposition of sulfamic acid and its derivatives by aprotic polar solvents, such as pyridine, N,N-dimethylformamide, and dimethyl sulfoxide, and, more recently, Kochetkov and associates[13] obtained sulfated polysaccharides of low sulfur content, almost without degradation, after treatment in aprotic polar solvents at 100°.

In order to develop a method for the selective liberation of *N*-sulfate groups in heparin, the decomposition of the pyridinium salts of 2-deoxy-2-sulfoamino-D-glucose and D-glucose 6-sulfate in polar solvents was studied.

EXPERIMENTAL

Analysis. — The content of amino groups was determined by the 2,4,6-trinitrobenzenesulfonic acid method[3], that of sulfate groups by turbidimetry[14], and that of D-glucose by colorimetry with glucose oxidase[15]. Paper electrophoresis was performed as previously described[4]. Thin-layer chromatography on Avicel SF was developed with two solvent systems: ethyl acetate–pyridine–acetic acid–water (5:5:1:3) and butyl alcohol–pyridine–water (5:4:3). The spots were detected with the ninhydrin[16] and alkaline silver nitrate reagents[17].

Preparation of the pyridinium salt of 2-deoxy-2-sulfoamino-D-glucose and D-glucose 6-sulfate. — The sodium salt of 2-deoxy-2-sulfoamino-D-glucose[18] and the commercial potassium salt of D-glucose 6-sulfate (Seikagaku Kogyo Co., Tokyo) were each passed through a column of Dowex 50W (X-8, H^+, 20–50 mesh), and the effluent was neutralized with pyridine and lyophilized. The thin film of pyridinium 2-deoxy-2-sulfoamino-D-glucose was washed first with ethanol and then ether to form an amorphous powder. The thin film of pyridinium D-glucose 6-sulfate was dried over phosphorus pentaoxide overnight at room temperature, and used as such.

Decomposition by polar solvents. — Pyridinium 2-deoxy-2-sulfoamino-D-glucose (~20 mg) or pyridinium D-glucose 6-sulfate (~75 mg) was weighed in a 5-ml volumetric flask and dissolved in the solvent to be tested. In the case of dimethyl sulfoxide containing methanol, the salts were first dissolved in a small volume of dimethyl sulfoxide, then the calculated amount of methanol was added, and finally dimethyl sulfoxide to bring the volume to the desired level. Aliquots of 0.5 ml of this solution were placed into Pyrex-glass tubes (1.3 × 10 cm), which were stoppered and warmed in a water bath at a constant temperature. At regular time intervals, a tube was removed, cooled in ice–water, and the content diluted with an equal volume of water to stop the reaction. The liberated inorganic sulfate was determined on a 0.2-ml aliquot of this solution. The amount of liberated amino groups and D-glucose residues was determined on 0.5 ml of this solution after suitable dilution.

Hydrolysis with 0.04M hydrochloric acid. — In a 5-ml volumetric flask, dry sodium 2-deoxy-2-sulfoamino-D-glucose (19 mg) or potassium D-glucose 6-sulfate (80 mg), both dried for 2 h at 80° *in vacuo*, were weighed accurately and dissolved in 0.04M hydrochloric acid. Aliquots (0.5 ml) of either solution were placed into Pyrex-glass tubes (1.3 × 10 cm), which were stoppered and heated at 100°. At regular time intervals, a tube was removed, cooled in ice–water, and the solution neutralized with 0.04M sodium hydroxide. The amount of liberated inorganic sulfate and amino groups and glucose residues was determined as just described.

RESULTS AND DISCUSSION

Nagasawa and Yoshidome[12] reported that, in the decomposition of various salts of cyclohexylsulfamic acid with hydrous pyridine, the decomposition rate of the pyridinium salt was the highest.

When pyridinium, ammonium, and triethylammonium salts of 2-deoxy-2-sulfoamino-D-glucose were treated with dimethyl sulfoxide containing 5% of water for 2 h at 50°, the liberation of both inorganic sulfate and 2-deoxy-2-amino-D-glucose, which were identified by paper electrophoresis and thin-layer chromatography, was 90%, 4%, and 0.5% for the three salts, respectively. Therefore, pyridinium 2-deoxy-2-sulfoamino-D-glucose was treated with five polar solvents that showed a great effect in the decomposition of cyclohexylsulfamic acid, *i.e.*, dimethyl sulfoxide, N,N-dimethylformamide, pyridine, acetonitrile, and hexamethyltriamide phosphate, all containing 5% of water. The decomposition rate of pyridinium 2-deoxy-2-sulfoamino-D-glucose was found to decrease in the order: pyridine > N,N-dimethylformamide > dimethyl sulfoxide > hexamethyltriamide phosphate > acetonitrile. While the decomposition was most rapid in pyridine and N,N-dimethylformamide, the 2-deoxy-2-amino-D-glucose formed was also decomposed (22% and 16%, respectively) after 7 h in these solvents. The value of the amino group content determined after treatment of the salt with hexamethyltriamide phosphate was abnormally high, whereas decomposition was slow in acetonitrile. Since pyridinium 2-deoxy-2-sulfoamino-D-glucose was decomposed fairly rapidly in dimethyl sulfoxide, whereas 2-deoxy-2-amino-D-glucose formed was stable under these conditions, dimethyl sulfoxide is the best suited polar solvent for the study of the decomposition of pyridinium 2-deoxy-2-sulfoamino-D-glucose. In any of the solvents just mentioned, the reaction became constant at ~95% degradation, and paper electrophoresis of the solution after completion of the reaction revealed the presence of a minute proportion of the starting 2-deoxy-2-sulfoamino-D-glucose. This phenomenon was also observed in the decomposition of cyclohexylsulfamic acid[12] and indicates that the reaction is reversible. Attempts to complete the *N*-desulfation by performing the reaction in the presence of barium chloride resulted in a decreased decomposition rate, which was 67% for 2 h at 50°.

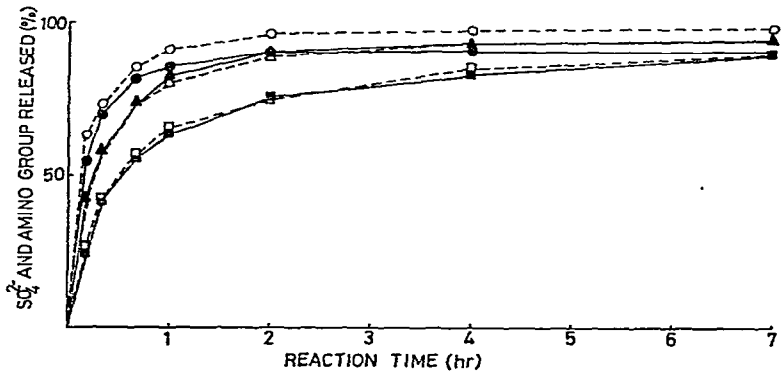

Fig. 1. Solvolytic desulfation of pyridinium 2-deoxy-2-sulfoamino-D-glucose in anhydrous dimethyl sulfoxide (○ – – – ○, amino group release; ●———●, sulfate release), 5% water–dimethyl sulfoxide (△ – – – △, amino group release; ▲———▲, sulfate release), and 10% water–dimethyl sulfoxide (□ – – – □, amino group release; ■———■, sulfate release) at 50°.

As shown in Fig. 1, treatment of 2-deoxy-2-sulfoamino-D-glucose pyridinium salt with anhydrous dimethyl sulfoxide and subsequent determination of the content of inorganic sulfate and amino groups indicate that the amount of amino groups liberated is 7–8% higher than that of inorganic sulfate. This observation suggests that the sulfate group has migrated from the amino to the hydroxyl group in 2-deoxy-2-sulfoamino-D-glucose, but a paper electrophoregram did not reveal a spot for the product of N→O trans-sulfation (mono-*O*-sulfated 2-deoxy-2-amino-D-glucose). Since the liberation of the amino and sulfate groups was the same in hydrated dimethyl sulfoxide, trans-sulfation would not take place in this case. The reaction rate for the liberation of sulfate groups decreased as the water content increased and the decomposition was entirely inhibited in the presence of over 25% of water.

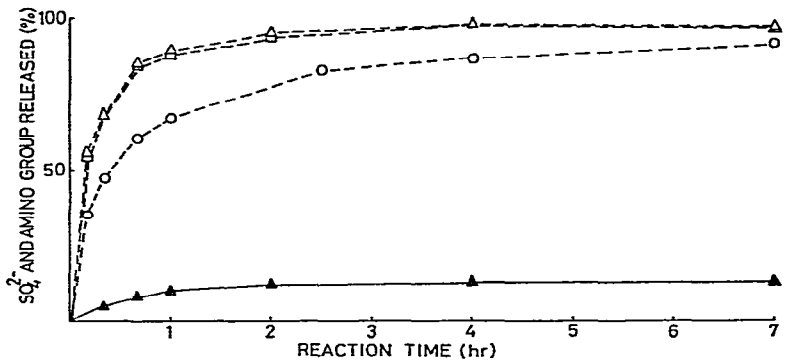

Fig. 2. Solvolytic desulfation of pyridinium 2-deoxy-2-sulfoamino-D-glucose in 5% methanol–dimethyl sulfoxide (△ – – – △, amino group release; ▲——▲, sulfate release), 10% methanol–dimethyl sulfoxide (□ – – – □, amino group release), and anhydrous methanol (○ – – – ○, amino group release) at 50°.

When pyridinium 2-deoxy-2-sulfoamino-D-glucose was treated with dimethyl sulfoxide containing methanol instead of water (Fig. 2), 2-deoxy-2-amino-D-glucose was liberated more rapidly than in dimethyl sulfoxide containing water, but the maximum value of the liberation of inorganic sulfate decreased to 35%, 13%, and 0%, with increasing concentration of methanol from 1% to 5% and 10%, respectively. Comparison of the n.m.r. spectrum of pyridinium 2-deoxy-2-sulfoamino-D-glucose in dimethyl sulfoxide-d_6 containing 10% of methanol immediately after dissolution with that obtained after the solution had been heated for 4 h at 50° shows that the peak of methyl proton of methanol at δ 3.24 had decreased and a new corresponding peak, due to the peak of methyl proton of methyl sulfate[19], had appeared at δ 3.47. This result indicates that, in dimethyl sulfoxide containing a small amount of methanol instead of water, *N*-desulfation results in the liberation of inorganic sulfate and methyl sulfate, and that methanolytic *N*-desulfation is predominant in dimethyl sulfoxide containing over 10% of methanol. Approximately the same *N*-desulfation took place in dimethyl sulfoxide containing ethanol.

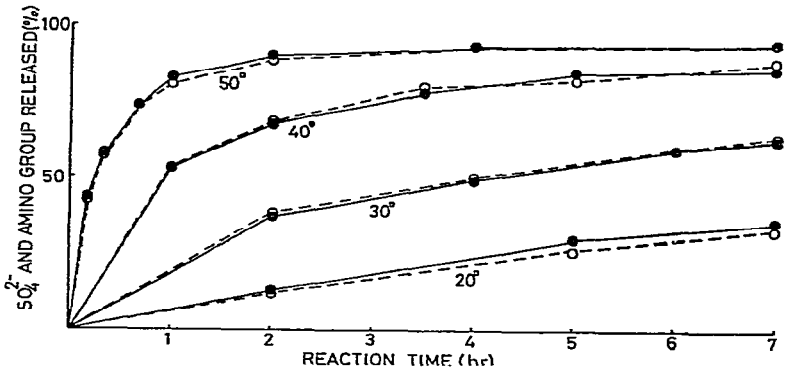

Fig. 3. Solvolytic desulfation of pyridinium 2-deoxy-2-sulfoamino-D-glucose in 5% water–dimethyl sulfoxide at various temperatures (○ – – – ○, amino group release; ●———●, sulfate release).

Examination of the decomposition of pyridinium 2-deoxy-2-sulfoamino-D-glucose in dimethyl sulfoxide containing 5% of water between 20° and 50°, shows an acceleration with the rise in temperature (Fig. 3).

Decomposition of pyridinium D-glucose 6-sulfate in anhydrous dimethyl sulfoxide and in dimethyl sulfoxide containing 5% of water or 5% of methanol is shown in Fig. 4. Comparison with the results obtained for pyridinium 2-deoxy-2-sulfoamino-D-glucose (Figs. 1 and 2) shows that both salts are more rapidly de-

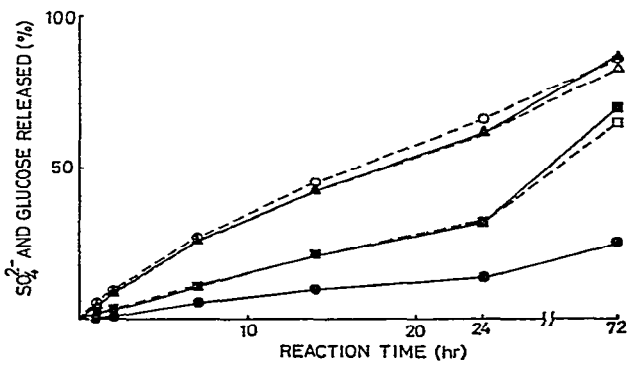

Fig. 4. Solvolytic desulfation of pyridinium D-glucose 6-sulfate in anhydrous dimethyl sulfoxide (△ – – – △, D-glucose release; ▲———▲, sulfate release), 5% water–dimethyl sulfoxide (□ – – – □, D-glucose release; ■———■, sulfate release), and 5% methanol–dimethyl sulfoxide (○ – – – ○, glucose release, ●———●, sulfate release) at 50°.

composed in dimethyl sulfoxide containing 5% of methanol than in the same solvent containing 5% of water. In dimethyl sulfoxide containing 5% of methanol, pyridinium 2-deoxy-2-sulfoamino-D-glucose and D-glucose 6-sulfate liberated, after 2 h, 95% of 2-deoxy-2-amino-D-glucose and 8% of D-glucose, respectively, whereas in dimethyl

sulfoxide containing 5% of water, they liberated 90% of 2-deoxy-2-amino-D-glucose and 3% of D-glucose, respectively, after 2 h. In the case of dimethyl sulfoxide containing 5% of methanol, difference in the liberation of D-glucose and inorganic sulfate corresponds to the formation of methyl sulfate.

It has been reported that *N*-sulfate groups are more rapidly hydrolyzed than *O*-sulfate groups under acid condition[4,20] but, as far as we are aware, decomposition of sugar *O*-sulfates under the conditions of the selective hydrolysis of the *N*-sulfate group in heparin, *i.e.*, with 0.04M hydrochloric acid for 2 h at 100°, has not been observed. Hydrolysis of sodium 2-deoxy-2-sulfoamino-D-glucose and of potassium D-glucose 6-sulfate in 0.04M hydrochloric acid at 100° gave the results shown in Fig. 5. It should be noted that even the 6-sulfate ester[21], the most stable sulfate ester among D-glucose sulfates, was hydrolyzed to 14% when hydrolysis of 2-deoxy-2-sulfoamino-D-glucose was almost complete.

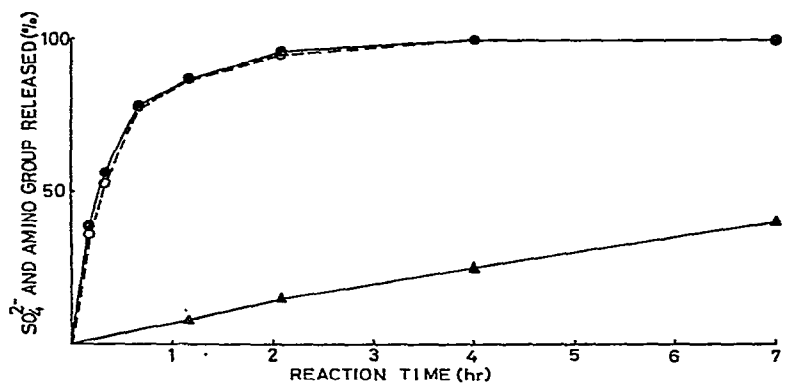

Fig. 5. Hydrolysis of sodium 2-deoxy-2-sulfoamino-D-glucose (○ – – – ○, amino group release; ●——●, sulfate release) and potassium D-glucose 6-sulfate (▲——▲, sulfate release) in 0.04M hydrochloric acid at 100°.

TABLE I

DESULFATION OF 2-DEOXY-2-SULFOAMINO-D-GLUCOSE AND D-GLUCOSE 6-SULFATE IN WATER–DIMETHYL SULFOXIDE, METHANOL–DIMETHYL SULFOXIDE, AND DILUTE HYDROCHLORIC ACID

Reaction conditions	*Liberation of 2-deoxy-2-amino-D-glucose or D-glucose* (%)		*Δ* (%)
	2-Deoxy-2-sulfoamino-D-glucose	*D-Glucose 6-sulfate*	
5% Water–dimethyl sulfoxide, 2 h, 50°	90	3	87
5% Methanol–dimethyl sulfoxide, 2 h, 50°	95	8	87
0.04M Hydrochloric acid 2 h, 100°	95	14	81

These results suggest that the solvolytic *N*-desulfation with dimethyl sulfoxide containing 5% of water or methanol is more selective for the liberation of *N*-sulfate groups than the present methods (Table I), and it seems possible to apply this method to the partial liberation of the sulfate groups in heparin.

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