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ANTICOAGULANT ACTIVITY OF SODIUM AMINODEOXYCELLULOSE SULFATES

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Synopsis

The anticoagulant activity of sodium aminopolysaccharide sulfates was tested *in vitro* by Lee and White method. All of them showed an activity in delaying coagulation of blood. Several sodium aminodeoxycellulose sulfates had higher anticoagulant activity than all of the commercial anticoagulants except for sodium heparinate.

Some films prepared by treating sodium aminodeoxycellulose sulfates with formalin or polyvinyl alcohol also had an anticoagulant activity.

INTRODUCTION

The most familiar property of heparin is a high activity as blood-anticoagulant, and is important for medical purposes¹⁻⁵). Mechanism of coagulation of blood is the conversion of soluble fibrinogen in blood into insoluble fibrin. This conversion is controlled by thrombin which is formed from prothrombin by catalysis with thrombokinase⁶). Several works⁷⁻¹⁰) on the syntheses of heparin analogue-polysaccharides (Heparinoid) have been reported. Whistler et al.¹¹) have stated that sodium salts of N- and O-sulfated chitosan and selectively N-sulfated chitosan were prepared and found to possess the high anticoagulant activity. Sodium aminodeoxycellulose sulfates obtained in a previous paper¹²) show the structural similarity to heparin and are expected to possess the anticoagulant activity.

Recently with the progress of medical science, many polymeric materials have become to be used for diagnosis and medical treatments¹³⁻¹⁵). The antithrombogenic materials have been developed for artificial organs. For example, Tanzawa et al.¹⁶) reported that the hydrophilic polyethylene glycol methacrylate and the quarternary ammonium salt of dimethylaminoethyl methacrylate were grafted

on the copolymer of vinyl chloride/vinyl acetate and the anticoagulant material was obtained by treatment of this graft copolymer with heparin. Sasaki¹⁷) reported that the insoluble antithrombogenic films were prepared by treatment of various anticoagulants with formalin or polyvinyl alcohol (PVA).

In this paper, the anticoagulant activity of sodium aminodeoxycellulose sulfates prepared in the previous paper¹²) is tested *in vitro* and some attempts are made to correlate the structure of the sulfates with their anticoagulant activity. The activity of films obtained by treatment of the sulfates with formalin or PVA in a similar way to the method of Sasaki¹⁷) is also tested *in vitro* in an attempt to develop new antithrombogenic materials from cellulose.

RESULTS AND DISCUSSION

In order to investigate the anticoagulant activity of sodium aminodeoxycellulose sulfates and sodium 6-amino-6-deoxystarch sulfate, the blood coagulation time in the presence of these compounds was measured by Lee and White method¹⁸). Experimental results with the concentration of 5 mg of the sulfates per 1 ml of blood are shown in Table 1. Sodium aminodeoxycellulose sulfates obtained by sulfation of aminodeoxycellulose and successive neutralization with sodium hydroxide

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Table 1 Anticoagulant activity of sodium aminodeoxycellulose sulfates.

Sodium Polysaccharide Sulfate	DS _{NH₂}	Abbreviation	Whole blood coagulation time (min) ^{a)} 5 mg/ml ^{b)}
Sodium aminodeoxycellulose sulfate	0.04	ACS _{0.04}	○
Sodium aminodeoxycellulose sulfate	0.08	ACS _{0.08}	○
Sodium aminodeoxycellulose sulfate	0.16	ACS _{0.16}	○
Sodium aminodeoxycellulose sulfate	0.68	ACS _{0.68}	○
Sodium aminodeoxycellulose sulfate	0.82	ACS _{0.82}	34.8
Sodium 6-amino-6-deoxycellulose sulfate	0.90	6ACS	○
Sodium 6-amino-6-deoxycellulose N-sulfate	0.90	6ACS-N	10.6
Sodium 2-amino-2-deoxycellulose sulfate	0.37	2ACS	○
Sodium 6-amino-6-deoxystarch sulfate	0.92	6ASS	○
Sodium chitosan sulfate	1.00		○
Sodium cellulose sulfate			29.5
Sodium heparinate			○
Control			5.7

^{a)} ○ ; No coagulation of blood after 2.5-hr elapse.

^{b)} Concentration: 5 mg of anticoagulant per 1 ml of blood.

were abbreviated to ACS_{0.04}, ACS_{0.08}, The subscripts of 0.04, 0.08, correspond to degree of substitution (DS) of amino group in original aminodeoxycellulose¹⁹⁾. The sodium sulfates prepared from 6-amino-6-deoxycellulose²⁰⁾, 2-amino-2-deoxycellulose²¹⁾, and 6-amino-6-deoxystarch²²⁾ were abbreviated to 6ACS, 2ACS, and 6ASS, respectively. Sodium salt of N-sulfated product of 6-amino-6-deoxycellulose was abbreviated to 6ACS-N. All of these sodium sulfates were readily soluble in water except for ACS_{0.68} and ACS_{0.82}.

The high anticoagulant activity was observed for ACS_{0.04}, ACS_{0.08}, ACS_{0.16}, ACS_{0.68}, 6ACS, 2ACS,

and 6ASS. The anticoagulant activity of 6ACS-N was very low, compared with that of 6ACS. This suggests that both O-sulfate and N-sulfate groups are necessary for a high anticoagulant activity. This agrees with the report of Whistler et al. that sodium chitosan O- and N-sulfates had a high anticoagulant activity whereas sodium chitosan N-sulfate did not. It is interesting that ACS_{0.68} insoluble in water possess a high activity because of the possibility of development of solid anticoagulant materials.

The anticoagulant activity of these salts was further investigated at lower concentration (Table

Table 2 Effect of concentration of sodium aminodeoxycellulose sulfate on blood-coagulation time.

Sodium Polysaccharide Sulfate	Whole blood coagulation time (min) ^{a)}	
	0.5 mg/ml	0.05 mg/ml
ACS _{0.04}	○	9.3
ACS _{0.08}	○	27.5
ACS _{0.16}	○	7.5
6ACS	○	8.9
2ACS	36.8	5.4
6ASS	53.7	5.5
Sodium chitosan sulfate	6.5	3.6
Sodium heparinate	○	

^{a)} ○ ; No coagulation of blood after 1.5-hr elapse.

2). ACS_{0.04}, ACS_{0.08}, ACS_{0.16}, and 6ACS showed a high activity at the concentration of 0.5 mg of anticoagulant per 1 ml of blood. However, 2ACS, 6ASS, and sodium chitosan sulfate showed a low activity at this concentration.

On the other hand, complete coagulation of blood was observed in 5 to 30 min. in the presence of lower concentration of 0.05 mg of anticoagulant per 1 ml of blood.

From the results described above, the following conclusions were deduced. For anticoagulation of blood, an anticoagulant has a threshold value. Moreover, ACS with low DS of sulfoamino group has as high activity as 6ACS with high DS of regioselective sulfoamino group. However, the anticoagulant activity of 6ACS is higher than that of 2ACS.

Table 3 shows many kinds of commercial anticoagulants²³⁾ and the contents of anticoagulant required to possess anticoagulant activity per 1 ml of blood. Some of sodium aminodeoxycellulose sulfates investigated above have higher activities than all of the commercial anticoagulants except

for sodium heparinate.

In order to determine the effect of functional group arrangement around the surface of solid anticoagulant materials, the anticoagulant activity of insoluble films which were obtained by treatment of sodium aminodeoxypolysaccharide sulfates with formalin or PVA in a similar way to the method of Sasaki¹⁶⁾, was tested *in vitro* by Lee and White method. Table 4 shows coagulation time of blood in a test tube of which inner wall was coated with the anticoagulant film. Films made from sodium sulfates (6ACS, 2ACS, and 6ASS) of aminopolysaccharides, which were obtained by regioselective amination of polysaccharides, remarkably prohibited blood coagulation. However, blood coagulation was observed for films made from ACS_{0.04}, ACS_{0.16}, and sodium cellulose sulfate in almost equal time to that for control films. It is interesting that sodium aminodeoxycellulose sulfates which showed high anticoagulant activity in blood solution did not give films with the activity. Probably, regular orientation of N-sulfate group at the surface of the films is a very

Table 3 Minimum contents required to possess anticoagulant activity per 1 ml of blood at various commercial anticoagulants.*

Anticoagulant	Structural formula	Minimum content per 1 ml of blood
Sodium heparinate		0.01 mg
Sodium citrate	$\begin{array}{c} \text{CH}_2 \text{COONa} \\ \\ \text{HOCCOONa} \cdot 2\text{H}_2\text{O} \\ \\ \text{CH}_2 \text{COONa} \end{array}$	5 mg
Sodium dextran sulfate		5 – 10 mg
Sodium oxalate	$\begin{array}{c} \text{COONa} \\ \\ \text{COONa} \end{array}$	1 – 2 mg
Potassium oxalate	$\begin{array}{c} \text{COOK} \\ \\ \text{COOK} \end{array}$	1 – 2 mg
Sodium fluoride	NaF	5 – 10 mg
Ethylenediaminetetraacetic acid (EDTA-2Na)	$\begin{array}{c} \text{NaOOCH}_2\text{C} \quad \quad \text{CH}_2 \text{COONa} \\ \quad \quad \quad \diagdown \quad \quad \diagup \\ \quad \quad \quad \text{N} - \text{CH}_2 - \text{CH}_2 - \text{N} \\ \quad \quad \quad \diagup \quad \quad \diagdown \\ \text{HOCH}_2\text{C} \quad \quad \text{CH}_2 \text{COOH} \end{array}$	1 mg
ACD liquid (acid citrate dextrose)	Citric acid, Sodium citrate, dextrose, and H ₂ O	5 mg
Double oxalate	Potassium oxalate, Ammonium oxalate, and H ₂ O	2 mg

*; Reference 23)

Table 4 Blood-coagulation time in a glass tube of which inner wall was coated with films prepared from 5% formalin or 2% PVA solutions containing sodium aminodeoxycellulose sulfate as anticoagulant.

Sodium Polysaccharide Sulfate	Whole blood coagulation time (min) ^{a)}	
	5% Formalin	2% Polyvinylalcohol
ACS _{0.04}	5.0	26.0
ACS _{0.16}	4.9	5.7
6ACS	○	○
2ACS	○	○
6ASS	○	○
Sodium chitosan sulfate	○	20.5
Sodium cellulose sulfate	3.8	30.5
Sodium heparinate	○	○
Control	2.7	12.3

^{a)} ○; No coagulation of blood after 1.5-hr elapse.

important factor in exhibiting high anticoagulant properties of solid materials. Such a factor is not necessary for the properties of sodium aminodeoxycellulose sulfates in blood solution. From these results, 6ACS, 2ACS, and 6ASS may be expected useful for the development of solid anticoagulant materials.

EXPERIMENTAL

Materials and method ---- The blood was obtained from the artery of dogs. Various sodium aminodeoxycellulose sulfates and 6-amino-6-deoxy-starch sulfate were used as anticoagulants.

A modification of the Lee and White method was used¹⁶⁾. The anticoagulant activity assay was carried out as follows: a solution of sodium aminopolysaccharide sulfate in water (0.1 ml) at a predetermined concentration in a glass test tube was warmed at 37°C in a water bath. Blood (1 ml) was drawn from the artery of a dog by a syringe. The blood was added into the anticoagulant solution in the test tube and kept at 37°C. The timer was started when blood was drawn by a syringe. The fluidity of the blood was checked at approximately every 30 sec interval shown in Fig. 1. When the blood completely coagulated, the coagulation time was measured. This test was continuously done for each of sodium aminopolysaccharide sulfates as the anticoagulant.

Preparation of anticoagulant films ----- A pyrex test tube which contained a solution of

Fig. 1 Assay for whole blood-coagulation time by test tube method (Lee and White method).

sodium aminopolysaccharide sulfate (2 mg) in 5% aqueous formalin (2–3 drops) or an aqueous PVA (2 mg in 0.1 ml of water) solution was swirled on heating in an oil bath at 80°C and then further heated at 80°C for 1 hr in a vacuum oven. Anticoagulant films were formed on an inner wall of the pyrex test tube. The change in fluidity of blood in the test tube was checked by Lee and White method as described above.

REFERENCES

- 1) M. Matsuda, M. Aoshima, N. Daitoh, K. Yamada, N. Tatsuta and Y. Kikasa, *Blood and Vessels*, **16**, 219 (1978).
- 2) E. W. Davie, K. Fujikawa, K. Kurachi, and W. Kiesel, *Advances in Enzymology*, **48**, 277 (1978).
- 3) J. G. Lemadam, S. Frye, Jr., and G. E. Phillips, *Clin. Chem.*, **12**, 263 (1966).
- 4) A. B. Foster and A. J. Huggard, *Advances Carbohydr. Res.*, **10**, 335 (1955).
- 5) L. H. Lam, J. E. Silbert, and R. D. Rosenberg,

- Biochem. Biophys. Res. Comm.*, **69**, 570 (1976).
- 6) Z. Yoshizawa, *Tanpakushitsu Kakusan Kooso*, **14**, 595 (1969).
 - 7) D. Horton and E. K. Just, *Carbohydr. Res.*, **29**, 173 (1973).
 - 8) R. L. Whistler and G. A. Towle, *Arch. Biochem. Biophys.*, **138**, 39 (1970).
 - 9) D. Horton and E. K. Just, *Carbohydr. Res.*, **30**, 349 (1971).
 - 10) M. L. Wolfrom and P. Y. Wang, *ibid.*, **18**, 23 (1971).
 - 11) R. L. Whistler and M. Mosik, *Arch. Biochem. Biophys.*, **142**, 106 (1971).
 - 12) T. Teshirogi, H. Yamamoto, M. Sakamoto, H. Tonami, *Sen-i Gakkaishi*, **36**, T-560 (1980).
 - 13) Y. Sakurai, *Igaku Kikai Zairyo*, **42** (12), 53 (1972).
 - 14) Y. Sakurai, *Kogyo Zairyo*, **25** (1), 25 (1977).
 - 15) K. Atsumi, *Naika*, **34** (4), 69 (1976).
 - 16) H. Tanzawa, *Kogyo Zairyo*, **25** (7), 68 (1978).
 - 17) S. Sasaki, Monbushoo Tokutee Kenkyu, 1978, p 113.
 - 18) S. Hino, "Rinsyo Kensa Koza", 15, Ketsueki-gaku, 1972, p 94.
 - 19) T. Teshirogi, H. Yamamoto, M. Sakamoto, H. Tonami, *Sen-i Gakkaishi*, **34**, T-510 (1978).
 - 20) *Idem.*, *ibid.*, **35**, T-525 (1979).
 - 21) *Idem.*, *ibid.*, **36**, T-501 (1980).
 - 22) *Idem.*, *ibid.*, **35**, T-479 (1979).
 - 23) Y. Yamaura, "Igaku Jikkenkooza", **13**, 1973, p 45.

硫酸化アミノデオキシセルロースの抗血液凝固性

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砺波宏明

アミノデオキシセルロース硫酸化物のナトリウム塩水溶液の抗血液凝固能をLee & White法で検討し、著しい効果をもつことを見い出した。

さらに、選択的アミノデオキシ化セルロースからのナ

トリウム塩とホルマリンあるいはポリビニルアルコール水溶液の混合物を熱処理し、内壁を薄膜でコーティングした試験管中では、血液は全く凝固しないことを見い出した。