Polysaccharide from Dura mater

By

Yoshiharu Saito and **Koh-Iti Turumi**

(齋藤 義治)

(鶴見 膠一)

*From the Department of Biochemistry, Fukushima
Medical College, Fukushima*

(Received for publication, October 10, 1954)

Chemical studies of dura mater has not as yet been accomplished. Histologically dura mater is composed of connective tissue. We examined the tissue histochemically by periodic acid oxidation method according to Hotchkiss¹⁾ and with positive result. Furthermore, metachromatic reaction with toluidine blue was given even at pH. 2.5 but commercial hyaluronidase (Sprase of Mochida Co.) showed no effect on the reaction, suggesting the presence of an acidic polysaccharide different from hyaluronic acid in this tissue. Therefore, it was attempted to isolate this acidic substance, and a new polysaccharide was obtained. From the analytical data it is assumed as a polysulphate of polygalacturonic acid. To add, it was found that this substance has an antihemocoagulative activity.

EXPERIMENTAL

2 g. of desiccated dura mater was freed from blood vessels, chopped and extracted with 20 ml. of 1 N sodium carbonate solution at room temperature (25–27°C.) for 24 hours. This procedure was conducted again and the combined extracts were neutralized with glacial acetic acid and dialyzed in tap water for about one week (12–15°C.). The solution was filtered, and the filtrate was concentrated under reduced pressure. From the concentrated solution crude substance was precipitated by addition of ethanol, and dried with ether. Yielded was about 500 mg. of a greyish-white powder here. The substance in this step gave positive biuret reaction. Therefore deproteinization was processed by Sevag method. Namely, 500 mg. of it was dissolved in about 50 ml. of water and shaken with a chloroform-octyl alcohol (9:1) mixture. After several repetitions of this procedure, a protein free, clear solution was obtained. It was concentrated under diminished pressure to about 15 ml. and precipitated with 4 volumes of ethanol. And the precipitate was collected by centrifugation and dried with ethanol and ether. A white powder given amounted to about 100 mg. This substance was electrophoretically homogeneous as

shown in Fig. 1.

Fig. 1. Electrophoretic pattern of polyuronide from dura mater in veronal buffer at pH 8.5. Current 16 mA, temp. 8°C., exposure : 38 min.

Qualitative and quantitative analyses are embodied in Table I.

TABLE I
Qualitative and Quantitative Properties of Dura
mater Polysaccharide

A. Qualitative test

Reaction	Result
Biuret	—
Ninhydrin	—
Molisch	—
Goldschmiedt	+
Naphthoresorcinol	+
Fehling:	
before hydrolysis	—
after acid hydrolysis	+
Hydrolysable sulfur	+
Phosphorus	—

B. Quantitative analyses

analysis	In per cent
Total nitrogen†	1.6-0
Reducing power* (as glucose) (after hydrolysis with 2 N H ₂ SO ₄ for 5 hours)	26.8-27.0
Hydrolysable sulfur‡	14.94
Ash	23.71

C. Optical rotation

$[\alpha]_D^{13}$	+1.3° (c=1, in water)
-------------------	-----------------------

† Micro-Kjeldahl method. * Hagedorn-Jensen's method. Reducing power reached maximum after 5 hours' hydrolysis. ‡ Saito's method²⁾.

Paper partition chromatography. 5 mg. of the substance were hydrolyzed with 1 N HCl for 3 hours in a sealed tube and then neutralized with sodium carbonate. After concentration under reduced pressure the hydrolysate

was sampled on a strip (Toyo filter paper No. 2). The chromatogram was developed in descending way according to Masamune and Yosizawa³⁾, employing *iso*-butanol-pyridine-water (1 : 1 : 1) as a solvent for irrigation. Developer, ammoniacal silver nitrate solution. Only one sugar spot corresponding to the position of galacturonic acid was detected. *p*-Anisidine reagent⁴⁾ was also used as an indicator, in which case the sugar spot showed the colour characteristic of uronic acid.

Test for antihemocoagulative activity. Since the substance obtained was a polysaccharide polysulphate ester, it was examined whether it has this activity or not by the method of Chargaff *et al*⁵⁾. As is shown in Table II, the coagulation time at a dilution of $1 : 4 \times 10^{-8}$ of the substance in the main run compared with that in the control.

TABLE II
Occurrence of Coagulation of Blood treated with *Dura mater* Polysaccharide

Dilution of the substance (1:)	Coagulation time	
4×10^{-2}	38 min.	00 sec.
4×10^{-3}	19	50
4×10^{-4}	15	50
4×10^{-5}	14	05
4×10^{-6}	11	25
4×10^{-7}	10	40
4×10^{-8}	7	05
Control blood	7	00
Citrated blood	no coagulation	

References

- 1) Hotchkiss, Arch. Biochem., 1948, **16**, 131.
- 2) Saito, Kagaku (Jap.), 1954, **24**, 46.
- 3) Masamune & Yosizawa, Tohoku J. Exp. Med., 1953, **59**, 1.
- 4) Mukherjee & Srivastava, Nature, 1952, **169**, 330.
- 5) Chargaff *et al.*, J. Biol. Chem., 1936, **115**, 149.