A low molecular weight glycosaminoglycan from the Human aorta[1]

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Summary. A low mol.wt, dialyzable glycosaminoglycan was isolated from human aorta and was found to be homogeneous on 2 dimensional electrophoresis. As judged by its electrophoretic mobilities and its hydrolysis by chondroitin sulfatase ABC, it was concluded that this hitherto unknown glycosaminoglycan is an oversulfated chondroitin sulfate.

The high mol.wt glycosaminoglycans of the arterial wall and other tissues have been extensively investigated and have been postulated to play important roles in maintaining the structural integrity of extracellular matrices. However, few studies have been directed toward the identification of other as yet undescribed glycosaminoglycans (GAGs). The present investigation was undertaken to isolate and partially characterize these hitherto unknown GAGs of the human aorta.

Materials and methods. Relatively normal human aortas which were obtained at autopsy were stripped of their adventitia and minced. After exhaustive delipidation and subsequent drying, the arterial tissue (in a typical experiment 3 g were used) was suspended in a pH 7.6 buffer and digested with 150 mg of recrystallized papain at 60 °C for 8 h. The obtained hydrolysate was centrifuged, and the supernatant solution dialyzed at 5 °C against 4 l of distilled water for 48 h. The resulting outside solution was concentrated by freeze-drying and applied to a Dowex 50-X2 (H-cycle) column (5 × 20 cm) which was then washed with 500 ml of water. The effluent containing the low mol.wt (lMW)-GAGs was concentrated and chromatographed on a Dowex 1-X2 (Cl-cycle) column (1.2 × 12 cm). After washing with 0.5 M NaCl, the GAGs were eluted stepwise by increasing the ionic strength of the eluting solution.

2-dimensional electrophoresis on cellulose acetate was carried out as described earlier[3,4]. Enzymatic hydrolysis of the lMW-GAGs was performed with chondroitinase ABC[5], and the extent of digestion was assessed by analysis of the resulting hydrolysate by this electrophoretic technique.

Results. Chromatography of the acidic, low mol.wt components of the arterial tissue on Dowex 1 yielded 4 fractions (figure 1). Only fractions 2[6] and 4 afforded the pink color characteristic for uronic acid by the Bitter-Muir reaction[7]. As judged by 2-dimensional electrophoresis (figure 2), the major fraction (peak 4, figure 1) contained a single GAG whose electrophoretic mobility did not correspond to those of authentic GAGs[3,5]. In the first direction of electrophoresis the lMW-GAG exhibited a mobility similar to that of heparin. However, in the second direction its mobility was greater than that of chondroitin sulfate C which exhibits the highest mobility

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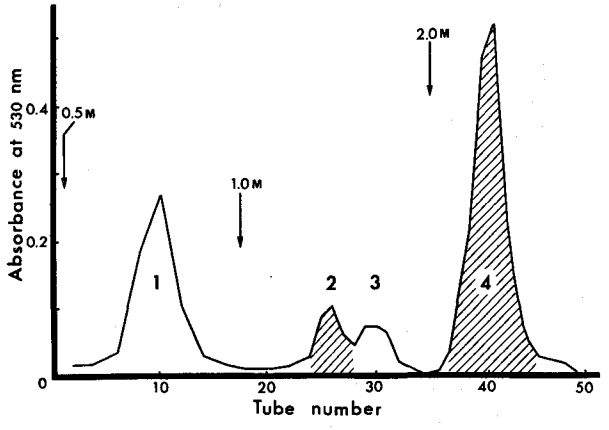

Fig. 1. Chromatography on a Dowex 1-X2 column of the low mol.wt, acidic compounds present in the proteolytic digest of the intima and media of the human aorta. The column was equilibrated with 0.5 M NaCl and subsequently developed stepwise by increasing the NaCl concentration of the eluant to 1.0 and 2.0 M. The resulting effluent was monitored for uronic acid by the method of Bitter and Muir[7]. The glycosaminoglycans were eluted in the fractions that are indicated by the dark areas.

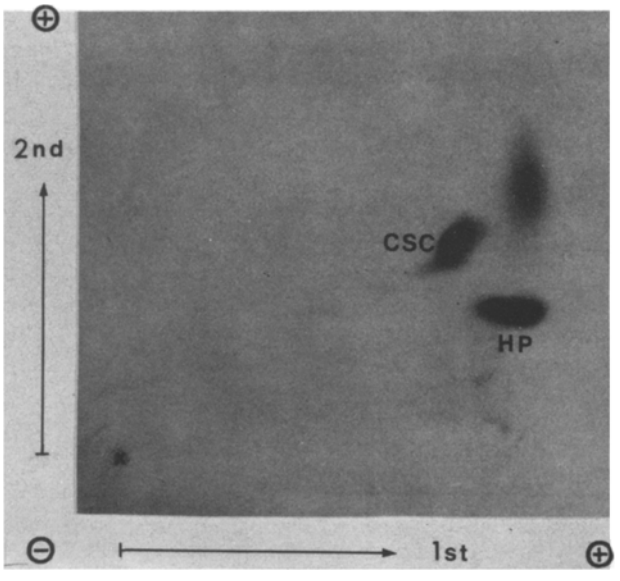

Fig. 2. 2-dimensional cellulose acetate electrophoresis of the lMW-GAG of the human aorta. The first direction of the electrophoresis (1st) was performed in pH 3.2 pyridine-formate buffer at 185 V for 70 min and the second direction of the electrophoresis (2nd) in 0.1 M $Ba(Ac)_2$ at 100 V for 6 h. As references chondroitin sulfate C (CSC) and heparin (HP) were applied at the same point of application (×) and run simoultaneously with the lMW-GAG.

of the known GAGs[3,5]. In enzymatic studies it was found that chondroitinase ABC hydrolyzed this mucopolysaccharide almost completely[8]. Further, this compound accounted for about 90% of the lMW-GAGs and for approximately 1% of the total GAGs[3] of the aorta.

Discussion. Little attention has been paid to the possible existence of lMW-GAGs in various tissues and fluids. To date because of the existing separation techniques, these compounds are overlooked and discarded during fractionation. However, with the availability of the new 2-dimensional electrophoretic procedure utilized in the present study, μg-quantities of GAGs can now successfully be investigated. Thus, we decided to modify the existing fractionation procedure and utilize this electrophoretic method to determine whether hitherto unknown lMW-GAGs exist.

As to the characterization of the isolated lMW-GAG, the digestion with a specific hydrolase (chondroitinase ABC) indicated that this GAG is a chondroitin sulfate. Moreover, the 2 electrophoretic mobilities of this compound suggest the presence of an oversulfated mucopolysaccharide.

The significance of an oversulfated lMW-chondroitin sulfate is difficult to assess on the basis of the available information. Although it could easily be speculated that the aortic lMW-GAGs may be due to incomplete degradation of the oversulfated portion of the high MW-GAGs or due to incomplete synthesis, it is nevertheless important to consider that this hitherto unknown GAG may in fact be a new entity requiring detailed chemical and biological analysis for its complete identification.

8 In control experiments carried out to assess the effect of papain and of incubation at pH 1–2 on high mol.wt GAGs, it could be established that to dialyzable mucopolysaccharides were formed.

Cynodontin, the tetrahydroxyanthraquinone of *Curvularia* and *Drechslera* species

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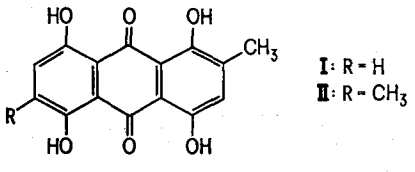
Summary. The red anthraquinone derivative isolated from several Drechslera and Curvularia species has been identified as cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone), and not as 1,4,5,8-tetrahydroxy-2,6-dimethylanthraquinone as reported earlier. Both pigments showed similar physico-chemical properties. A good distinction could be obtained by gas-liquid chromatography.

In the course of a study on the production of metabolites, especially anthraquinone pigments, produced by species of Drechslera, we always identified the isolated red tetrahydroxyanthraquinone as cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone) (**I**), but found never 1,4,5,8-tetrahydroxy-2,6-dimethylanthraquinone (**II**), the pigment reported by Bohlmann et al.[1] for Curvularia lunata (Wakker) Boedijn (st. asc. Cochliobolus lunatus Nelson et Haasis), and subsequently for Drechslera spicifera (Bain.) v. Arx (st. asc. C. spicifer Nelson)[2], D. sorokiniana (Sacc.) Subram. et Jain [st. asc. C. sativus (S. Ito et Kuribayashi) Drechsler ex Dastur][3] and also several other Curvularia species[2]. Since the dimethylanthraquinone is somewhat unusual[4] and provides the only example of an anthraquinone in which C-methylation would occur in its biosynthesis[5], we decided to make more intensive examinations of the existence of this pigment as a natural substance.

The original natural **II** was not available[6] and, therefore, we re-isolated the pigment from cultures of the C. lunata strain used by Bohlmann and his co-workers. The isolated product was compared with natural cynodontin (**I**) and synthetic **II**, which we synthesized according to Flumiani[7]. The identity of the synthetic product was confirmed by high resolution mass spectrometry (MS).



Curvularia lunata NRRL 2380[8] was cultivated in a medium containing peptone (microbiotone Oxoid) 5 g, glucose 50 g, $MgSO_4 \cdot 7\ H_2O$ 0.25 g and K_2HPO_4 0.25 g per liter deionized water. Erlenmeyer flasks of 300 ml, each containing 100 ml of medium, were incubated as still cultures at 24 °C for 18 days. Mycelium collected from 9 flasks was extracted with ethyl acetate using a Waring blendor. After evaporation of the solvent, the residue (447 mg) was treated with light petroleum, bp 40–60 °C, for removal of the fatty material. The remaining material (21 mg) was recrystallized from ethyl acetate. Brown crystals (4 mg) with a bronze lustre, designated as compound **III**, were obtained. Dry weight of the mycelium after extraction was 4.4 g.

Drechslera halodes (Drechsler) Subram. et Jain CBS 273.52 was grown as surface cultures on Czapek Dox medium supplemented with 0.05% yeast extract (Oxoid) and 1 ml/l medium of a trace elements solution, prepared according to Kitto et al.[9], for 14 days at 24 °C. Mycelium from 6 Erlenmeyer flasks (100 ml medium/flask) was collected and also extracted with ethyl acetate. Removal of the solvent followed by washing with light petroleum

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