Journal of Thermal Analysis, Vol. 1 (1969) 241–245

THERMAL INVESTIGATIONS ON NATURALLY BOUND GLYCOSAMINOGLYCAN COMPLEXES

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(Received March 27, 1969)

A derivatograph has been used successfully for the investigation of glycosaminoglycan, proteins, as well as their lipid complexes in native biological tissues. Details and discussion of these problems are available in the papers cited in the references.

Thermoanalytical methods are widely used for the structural investigation of inorganic compounds, but very few studies are available on the thermal properties of naturally occurring organic materials. In our current experiments, derivatograph has been used successfully to study the glycosaminoglycans (GAG-s) and the proteins of connective tissue, in their natural complexes.

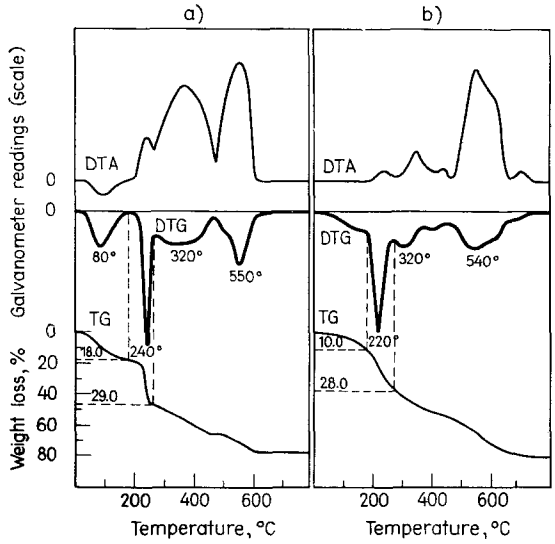

Fig. 1. *a)* Chondroitin sulphate; *b)* Human aortic glycosaminoglycan

The studies were carried out using a Paulik—Paulik—Erdey MOM Derivatograph [1]. The samples (approximately 100 mg) were weighed into a platinum crucible. The heating rate was 10°/min up to 900°.

When plotting the rate of weight change as a function of temperature, a peak was found between 240–270° on the derivative-thermogravimetric (DTG) curves, characteristic for a series of GAG-s (chondroitin sulphate, hyaluronic acid, heparin, GAG-mixtures prepared from aorta intimas of different species), accompanied by a weight loss of the same order [2].

Fig. 1 shows typical derivatograms obtained for these samples. In Fig. 1*a* thermal decomposition curves of chondroitin sulphate are presented. The moisture content of the sample, that is released at a maximum rate at 80° is 18 per cent according to the thermogravimetric (TG) curve. The sample is thermostabile up to 200°. Decomposition starts at 200° and reaches its maximum rate

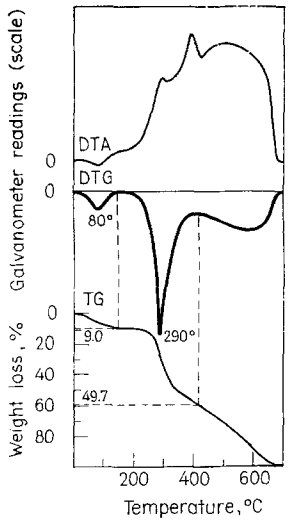

Fig. 2. Elastin

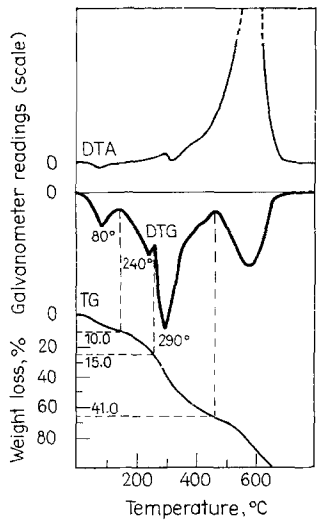

Fig. 3. Healthy human aorta intima

at 240°. This process is accompanied by a weight loss of about 29 per cent. With increasing temperature, decomposition continues at a constant rate and two additional DTG maxima are formed at 320° and 550°. The previous decomposition is combined with an exotherm enthalpy change (differential thermal analytical DTA curve). It might be concluded that the enthalpy change is determined by the recombination and burning of the compounds formed during the decomposition process. Fig. 1*b* shows the derivatogram of a human aortic GAG-preparation. There is a sharp peak on the DTG curve at 220°, accompanied by 28 per cent weight loss. At 320° and 540° two additional maxima appear.

In the thermal behaviour of the main protein components of connective tissue (elastin, collagen) a similarity was also established.

Fig. 2 presents the thermal decomposition curves of elastin as an example. It can be seen that after the evaporation of moisture, decomposition of the sample

starts at 200° and the maximum rate is at 290° according to the DTG curve, accompanied by a weight loss of 49.7 per cent [3].

The characteristic GAG and protein peaks were also detectable on the DTG curves of young, healthy human aorta intimas, indicating the presence of these components in the aortic tissue. The decomposition of chemical bonds in GAG and protein was not affected by their appearance in biological environment [4].

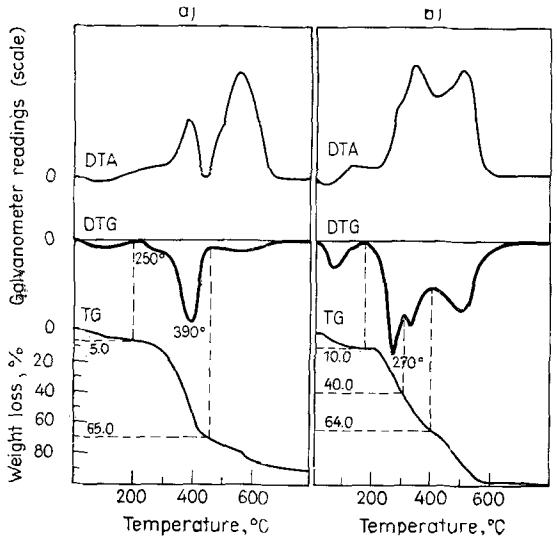

Fig. 4. *a)* Glycosaminoglycan-β-lipoprotein complex; *b)* Delipidated glycosaminoglycan-β-lipoprotein complex

A derivatogram of a human aorta is presented in Fig. 3. Water content first disappears, and decomposition starts at about 200°. According to the DTG curve, the first process, that reaches its maximum rate at 240°, results in 15 per cent weight loss. (This peak was found to be characteristic for the decomposition of GAG-s.) The main reaction leading to 41 per cent weight loss, occurs at 290° with maximum rate as a result of the decomposition of structural proteins.

The change in the amount of components decomposed at the temperatures found characteristic for GAG-s and proteins in the aorta could be followed by this method.

In coronary atherosclerosis lipids of the serum are deposited in the aorta intima, thus leading to the formation of atherosclerotic plaques. Based on the results of our previous in vitro investigations it was suggested that the GAG-s of the connective tissue of the arteries may in some way render lipids of the blood insoluble and so set the stage for the development of the lesions of atherosclerosis.

By using the thermal method, the existence of the suggested GAG-beta-lipoprotein (GAG-βLP) complexes in the aortic tissue could be proved.

Fig. 4*a* shows typical thermal decomposition curves for the in vitro aortic GAG-serum-beta-LP complex. It might be concluded that the incorporation of the lipoprotein moiety into the molecule resulted in an increase of thermostability. Decomposition took place with maximum rate only at 390°, according to the DTG curve; the characteristic peak found on the DTG curves of GAG preparations at about 240° diminished here to an inflexion.

The derivatogram of the delipidated in vitro GAG-β-LP complexes is shown in Fig. 4*b*. It is remarkable that after the extraction of lipids the maximum at

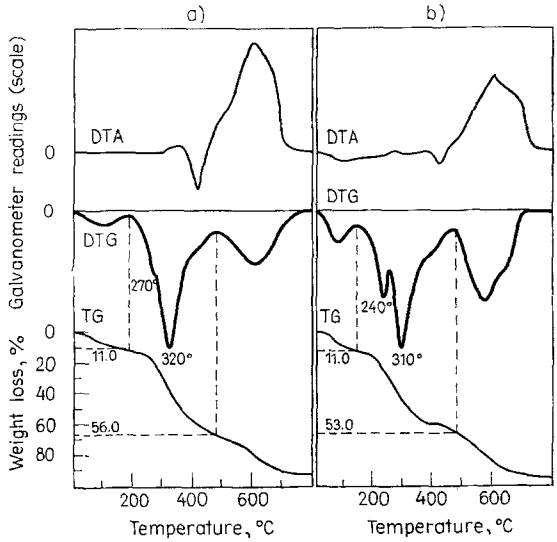

Fig. 5. *a)* Atherosclerotic human aorta intima; *b)* Delipidated atherosclerotic human aorta intima

270° reappears on the DTG curves, which is followed by a smaller weight change at 330°, caused by the protein moiety of the LP.

The curves obtained when investigating average samples of atherosclerotic aortas resembled those of the in vitro GAG-β-LP complexes. In the delipidated atherosclerotic intimal tissue the 240—270° peak reappeared again on the curves, producing the same picture as in the case of dissociated in vitro complexes (Fig. 5*a*, *b*).

Based on the results reported above it can be concluded that

1. thermal investigation is a useful method for the analysis of biological materials, particularly for GAG-s, proteins and their complexes, as this method is based on the decomposition of naturally occurring compounds of macromolecules;

2. the characteristic peaks appearing on the DTG curves of various GAG and protein preparations and biological tissues containing these substances,

indicate that those types of chemical bonds which are not influenced by the presence of other structural linkages were decomposed at 240° and 300° in the GAG and protein molecules;

3. when these compounds are converted into complexes containing lipid (in vitro, or as a result of a pathological in vivo process) an increase of thermostability was established. This phenomenon could be used to prove the existence of GAG-β-LP complexes in the atherosclerotic aorta, emphasizing the role of GAG-s in the mechanism of lipid deposition in the atherosclerotic process.

References

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3. J. SIMON, M. BIHARI-VARGA and L. ERDEY, Acta Biochim. et Biophys. Acad. Sci. Hung., (in the press).
4. M. BIHARI-VARGA, J. SIMON and S. GERŐ, Acta Biochim. et Biophys. Acad. Sci. Hung., 3 (1968) 375.

RÉSUMÉ — La méthode de la dérivatographie a été appliquée avec succès à l'examen de Glycoseaminoglycane de protéines et leurs complexes avec lipides dans des tissus natifs biologiques.

ZUSAMMENFASSUNG — Der Derivatograph wurde zur Untersuchung von Glycoseaminoglycan, Proteinen und ihrer Lipidkomplexen auch in nativen biologischen Stoffen mit Erfolg angewandet. Näheres und Besprechung dieser Probleme sind in den in der Literatur angegebenen Veröffentlichungen zu finden.

Резюме — Методом дериватографии исследованы гликозаминогликаны, протеины и их липоидные комплексы, входящие в состав биологических тканей. Эта проблема дискутируется в работах, приведенных в ссылках.