Journal of Thermal Analysis, Vol. 38 (1992) 153-157

THE APPLICATION OF THERMOANALYTICAL METHODS IN STUDIES ON THE PATHOMECHANISM OF HUMAN DISEASES

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This mini-review attempts to give a short summary of the application of temperature-dependent techniques in bio-medical research. Results obtained by thermal analysis provide valuable support to scientific theories on various human pathophysiological processes as well as to several therapeutic approaches. Special attention is focussed on cardiovascular disease, since of approx. 11 million deaths occurring per annum in developed countries more than 3.9 million were due to this disease, as recorded by WHO. Recent research on the pathogenesis of atherosclerosis has concentrated on the physico-chemical nature of the arteries and on the roles of serum lipoproteins and defects in cellular metabolism of cholesterol. An understanding of the physical and chemical nature of lipids accumulated in the arterial wall may help explain how it gets into the artery and how it can be removed. Smectic cholesteryl-ester phases had been observed in a number of fatty streaks and advanced atherosclerotic lesions within the arteries. These phases have physical characteristics that can be identified with the polarizing or electron microscope, by calorimetry, by NMR spectrometry or by X-ray diffraction.

Keywords: bio-medical research, pathomechanism, thermoanalytical methods

Introduction

It is generally known that temperature-dependent techniques can successfully be used in the diagnosis of various diseases (for examples see Table 1), or in the quality control and optimization of therapeutic activity.

Far less information is available on their application in basic medical or biological research, may be with the exception of studies concerning the maturation, ageing, pathological alterations and repair of structural proteins (collagen, elastin) of the various organs.

Recent research on the pathogenesis of atherosclerosis has focused on the physicochemical nature of the arterial wall. A striking feature of

John Wiley & Sons, Limited, Chichester
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atherosclerosis is the accumulation of large amounts of lipids in the arterial intima in the form of various types of atherosclerotic plaques. Most of the lipid in the plaque derives from the blood where it is circulating in the form of water-soluble lipoproteins. An understanding of the physical and chemical nature of this accumulated lipid may help explain how it gets into the intima and how it can be removed. In this paper the structural features of the lipoproteins involved in atherosclerosis will be reviewed, with special reference to the characteristics that can be identified with thermoanalytical methods.

Table 1 Examples for the diagnostic application of thermoanalytical methods

Method	Disease
Thermography	Acute pancreatitis
	Atherosclerotic aorta
	Breast cancer
	Cholesteomatoses
	Chronic disease of duodenum
	liver
	stomach
	Malignant melanoma
	Myocardial ischaemia
	Nerve lesions
	Oral lesions
	Rheumatoid arthrosis
	Testicular diseases
	Varicocele
Thermogravimetry	Composition of urinary stones
	Hydration of actin
	lenses
	salivary glands
Calorimetry	Ichthyosis of stratum corneum
	Tracheal mucus rheology

Serum low-density lipoprotein (LDL) is the major lipoprotein class involved in cholesterol transport. Many epidemiological studies have shown that coronary heart disease is positively correlated with LDL levels.

LDL is a quasi-spherical particle with most of the polar lipids (cholesterol, phospholipids) and apolipoprotein-B molecule forming the shell and covering the apolar constituents (cholesteryl esters, triglycerides) located in the centre of the particle. Depending on the temperature of the sample, esterified cholesterol may be in one of three stages: a liquid state, in

which the molecules are randomly associated, a smectic liquid-crystal state, in which the molecules form a layered structure perpendicular to the long axis; or crystalline solid state. Each of these phases has physical characteristics: if liquid, it is isotropic; the liquid crystalline phase appears in the form of birefringent spherulites under the polarizing microscope. Each ester has a well-defined transition temperature that can be quantified by differential scanning calorimetry (DSC) or determined on the basis of the variation of the methylene resonances by NMR spectrometry.

Using the above temperature dependent techniques a reversible transition in LDL encompassing body temperature has been defined, which is associated with an order-disorder liquid crystalline phase change of cholesteryl esters within the LDL particle [1]. For normal LDL the transition is below body temperature [2], i.e. LDL molecules exist in a fluid state at physiological conditions.

In certain types of dyslipoproteinemias, generally considered as one of the most important risk factors for atherosclerosis, the concentration of LDL in the serum is significantly increased. The idea, that quantitative changes in LDL content might initiate qualitative alterations in the structure of the LDL molecule, seemed to be concievable.

Results of studies of LDL from the hyperlipemic rhesus monkey [3], from cholesterol fed rabbits [4] and from type IIa hyperlipemic patients [5] demonstrated the elevation of the characteristic phase-change temperature and revealed increased amounts of ordered structures attributed to the presence of cholesteryl esters in smectic-like mesophases (Table 2).

Table 2 Liquid crystalline to liquid phase transition temperatures of serum LDL

Source of serum	Thermal transition midpoint /°C		Polarizing light microscopy
	DSC	NMR	
Normal rabbit	37.8	38.0	37.5–38.5
Hyperchol. rabbit	40.3	39.8	39.5–40.6
Normal human subject	34.2	34.8	34.0–34.9
Type IIa homozygote	37.8	38.4	37.5–39.0

The existence of liquid crystalline LDL, or some of its subpopulations [6] in hyperlipemic serum may be causally related to atherogeneicity: smectic core cholesteryl esters may limit the ability of lysosomal enzymes to degrade LDL and limit the transfer of lipids, thus interfering with normal metabolic processes.

Plasma LDL has been shown to be selectively retained in the atherosclerotic lesions of human aortas. Serum-derived LDL present in the lesions is known to have undergone some structural modifications as well. Several investigators have studied the physical structure of these deposits and detected sharp thermotropic order-disorder transitions in human aortic samples containing fatty streaks, which is consistant with the presence of smectic cholesteryl ester domains [7, 8]. No evidence was found for similar order-disorder transitions in normal aortae. The question, whether this structural alteration of the LDL molecule takes place already in the blood or after entering the vascular wall was approached by studying the physical structure of LDL isolated from the serum and from the aortic intima of atherosclerotic individuals. The results presented in Table 3 demonstrate that in each case serum LDL showed normal thermal transitions and was in a fluid state around body temperature, whereas LDL isolated from the intimal lesions revealed elevated phase change temperatures and appeared in the form of liquid crystals at physiological temperatures.

Table 3 Liquid crystalline to liquid phase transition temperatures of LDL isolated from the eserum and from the aortic intima of the same atherosclerotic subject

Source of serum	Thermal transition midpoint /°C		Polarizing light microscopy
	DSC	NMR	
Subject 1: serum	33.5	34.5	33.0–34.8
aorta	40.8	41.5	40.0–42.2
Subject 2: serum	34.6	34.8	33.5–35.8
aorta	41.0	41.6	39.6–42.0
Subject 3: serum	34.7	35.0	34.0–35.8
aorta	40.9	41.3	39.9–42.3

There is still divergence of opinion about the mechanism underlying the stages of the process in relation to the structural alteration of LDL within the arterial wall. In the last three decades direct and indirect studies proved that in the course of atherogenesis LDL might be bound specifically to the protein-polysaccharide macromolecules (porteoglycans, PG) of the extracellular matrix of the arterial wall. The investigation by DSC or NMR spectroscopy of complexes prepared by the addition of PG-s isolated from the arterial wall of various species to serum LDL, demonstrated the formation of liquid crystalline structures [9, 10]. The phenomena observed in model systems may also take place in vivo. The formation of PG-LDL complexes within the

arteries may result in the establishment of liquid crystalline LDL structure and may play a role in the immobilization of LDL and in the development of the atherosclerotic lesion.

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Zusammenfassung — Es wird versucht, einen kurzen Überblick über die Anwendung temperaturabhängiger Techniken in der biomedizinischen Forschung zu geben. Thermoanalyti-sche Ergebnisse bieten eine wertvollen Beitrag für wissenschaftliche Theorien zu verschiedenen pathophysiologischen Vorgängen beim Menschen als auch für verschiedene therapeutische Methoden. Besondere Aufmerksamkeit wird Herzkreislaufkrankheiten geschenkt, da laut WHO-Berichten von den jährlich etwa 11 Millionen Todesfällen in den hochentwickelten Ländern mehr als 3,9 Millionen auf diese Krankheiten zurückzuführen sind. Die jüngste Forschung auf dem Gebiete der Pathogenese von Atherosklerose konzentrierte sich auf die physikalisch-chemische Natur der Arterien sowie auf die Rolle von Serumlipoproteinen und Defekte im Zellmetabolismus von Cholesterol. Eine eingehende Untersuchung der physikalischen und chemischen Natur von den in der Arterienwand angehäuften Lipiden kann helfen zu erklären, wie diese in die Arterie gelangen und wie sie von dort entfernt werden können. In einer Anzahl von fettigen Bändern und krankhaften atherosklerotischen Veränderungen im fortgeschrittenen Stadium in den Arterien wurden smektische Cholesterylesterphasen beobachtet. Diese Phasen besitzen physikalische Eigenschaften, die mittels Polarisations- oder Elektronenmikroskop, Kalorimetrie, NMR-Spektroskopie und Röntgendiffraktion identifiziert werden können.