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Differential scanning calorimetry study of blood serum in chronic obstructive pulmonary disease

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Abstract Differential scanning calorimetry (DSC) has been applied for studies of blood serum from patients sick with chronic obstructive pulmonary disease (COPD). The denaturation of serum proceeds as endothermic process over the temperature range 45–85 °C. Distinct changes in the shape of DSC curves have been observed for serum from patients with severe stage of COPD (treated with inhaled corticosteroids) relative to serum from healthy individuals. The first moment of the thermal transition with respect to the temperature axis shifts from the normal value of 63.9 ± 0.3 to 65.3 ± 0.7 °C and to 67.6 ± 1.6 °C for early and advanced stages of disease, respectively. The results of our studies suggest age dependence of blood serum denaturation transition.

Keywords Chronic obstructive pulmonary disease · Differential scanning calorimetry · Protein denaturation · Serum

Introduction

Differential scanning calorimetry (DSC) has appeared to be a useful and well-applicable method in the research of various medical problems [1–3]. Serum is a very informative sample for disease diagnosis. According to recent reports, DSC studies of plasma or serum can be applied for disease detection and monitoring [4–8]. It has been evidenced that plasma as well as serum DSC curve from diseased individuals differ dramatically from normal individuals. Garbett et al. [8] investigations have shown that plasma from healthy individuals yields a reproducible signature thermal curve although sensitive to ethnicity and gender. Each disease (e.g. rheumatoid arthritis, lyme disease, systemic lupus, cervical cancer, lung cancer, ovarian cancer) seems to display a signature thermal transition that can at a glance be distinguished from other diseases [6–8].

In the present study, serum of patients sick with chronic obstructive pulmonary disease (COPD) has been investigated using DSC method. COPD is a progressive disease that makes it hard to breathe. COPD is comprised primarily of two related diseases—chronic bronchitis and emphysema. COPD can cause coughing that produces large amounts of mucus, wheezing, shortness of breath, chest tightness and other symptoms. The primary risk factor for COPD is chronic tobacco smoking. Long-term exposure to other lung irritants, such as air pollution, chemical fumes, or dust, also may contribute to COPD. Other risk factors include: age, heredity, a history of childhood respiratory infections. COPD is more common in men than women.

Materials and methods

Serum samples

Serum came from the blood of 10 patients with COPD (1 woman, 9 men; 40–73 years old, all smoke or used to smoke over 19–43 years) and 5 healthy men (49–55 years old; 2—non-smokers and 3—smokers). Among patients with COPD 5 of them were with early stage of disease and

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5 with severe stage. Patients with severe COPD were treated with inhaled corticosteroids.

The blood was dissolved in HBSS buffer (Hanks' Balanced Salt Solution) in proportion: 1 mL of blood, 2.5 mL of HBSS. The obtained serum samples were diluted tenfold in degassed water pro injection. The final pH of serum solution was 7.5 ± 0.1.

Protein concentration was determined according to its dry mass in serum sample.

Differential scanning calorimetry

DSC scans were performed on an VP DSC MicroCal instrument (Northampton, MA) from 20 to 95 °C at 1 °C min^{-1} scan rate with a pre-scan equilibration time of 15 min. Thermal curves were plotted as Excess Specific Heat Capacity, C_p^{ex}, (J $°C^{-1}$ g^{-1}) versus temperature. Sample scans were first corrected for the instrumental baseline and next by applying a linear baseline fit. Scans were finally normalised for the gram mass of protein. Data were analysed using Origin 7.0.

Results and discussion

Serum DSC curves for healthy individuals

Because most of the time, COPD is diagnosed in middle-aged or older people, the serum of healthy individuals of age above 49 was taken as control. DSC curves of serum from 5 healthy individuals were collected and averaged to yield an average thermal curve for healthy serum (Fig. 1).

The denaturation of serum proceeds over the temperature range 45–85 °C. The complex endothermic DSC transition observed for serum arises from the denaturation of the constituent proteins and represents the weighted sum of denaturation profiles of the individual proteins within

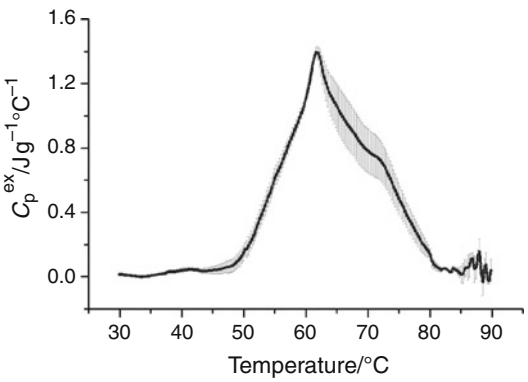

Fig. 1 Averaged DSC curve of serum from healthy individuals. The *shaded area* is the standard error of the mean (SEM) at each temperature

serum. The major peak at about 62 °C primary reflects the denaturation of unligated HSA, with a contribution from haptoglobin. The shoulder at about 70 °C arises primarily from IgG and non-defatted albumin. The average serum curve obtain for our control group is very similar to the curve reported for normal plasma by Garbett et al. [7, 8]. However, minor differences can be observed. These differences are due to fibrinogen absence in serum, the kind of solvent used for serum dissolution and probably also the age of volunteers from control group.

In Table 1 the thermodynamic parameters: the temperature of peak maximum (T_m), the specific enthalpy (ΔH) of serum denaturation (calculated as the area under the thermogram) and the width of peak in its half height (HHW) are shown. Additionally, the ratio of C_p at 70 and 62 °C and the normalised first moment (M_1) of the thermal transition with respect to the temperature axis calculated as:

$$M_1 = \frac{\int_{T_1}^{T_2} TC_p dT}{\int_{T_1}^{T_2} C_p dT} \quad (1)$$

where T_1 and T_2 are the initial and final transition temperatures, respectively, are presented. The value of T_m = 61.9 ± 0.2 °C obtained in our study, is slightly lower than 62.8 °C, reported by Garbett et al. [7]. M_1 = 63.9 ± 0.3 °C characterising our control group is below the value 67.4 ± 0.8 °C found for plasma of 15 healthy individuals 22–50 years old [7]. The enthalpy of serum (plasma) denaturation obtained by us and Garbett et al. is practically the same: 5.32 ± 0.64 and 5.02 ± 0.32 cal g^{-1}, respectively.

DSC curves for disease states

In Fig. 2 the averaged DSC curve for patients with COPD is shown. When this curve is compared with averaged one for healthy individuals it is apparent that there is a decrease in the amplitude of the major peak at ~62 °C with concomitant increase in amplitude of the shoulder at ~70 °C. The ratio C_p^{70}/C_p^{62} increases from 0.55 for control to 0.95 for COPD group. The increase of mean T_m and M_1 values for COPD serum indicates the shift of transition towards higher temperatures. As can be seen from Table 1, the above effects are even more pronounced for serum from patients with severe stage of COPD, treated with inhaled corticosteroids.

The curves shown in Fig. 3 for different stages of a disease compared to normal serum indicate that the averaged thermal curve obtained for patients with early stage of disease is only slightly abnormal while that for patients

Table 1 The thermodynamic parameters (mean ± standard deviation; in parenthesis—the values found for averaged curves) of serum denaturation transition

Serum	T_m/°C	ΔH/J g^{-1}	HHW/°C	M_1/°C	C_p^{70}/C_p^{62}
Control	61.9 ± 0.2 (61.8)	22.3 ± 2.7 (23.0)	14.1 ± 2.3 (16.5)	**63.9 ± 0.3** (64.1)	0.55 ± 0.08 (0.56)
COPD	64.3 ± 1.4 (61.9)	20.3 ± 1.1 (20.0)	15.9 ± 0.8 (18.3)	**66.5 ± 0.9*** (65.9)	0.95 ± 0.18 (0.80)
Early COPD	62.2 ± 0.3 (62.5)	20.4 ± 1.9 (20.4)	16.0 ± 0.8 (16.5)	65.3 ± 0.7 (65.3)	0.68 ± 0.06 (0.67)
Severe COPD	66.4 ± 2.5 (62.1)	20.3 ± 1.4 (20.4)	15.9 ± 1.5 (19.1)	**67.6 ± 1.6*** (67.5)	1.22 ± 0.33 (1.00)

* Significant differences in comparison with control group ($p < 0.05$)

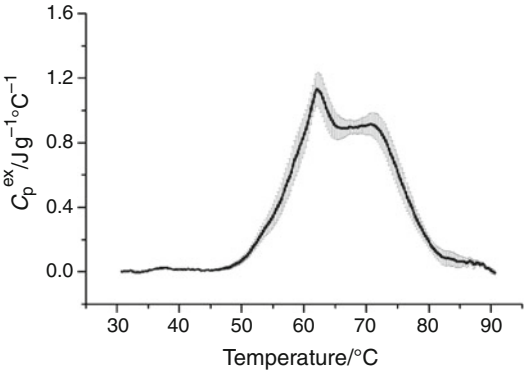

Fig. 2 Averaged DSC curve of serum from patients with COPD. The *shaded area* is the standard error of the mean (SEM) at each temperature

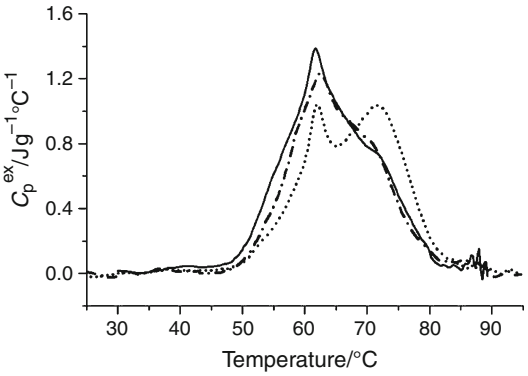

Fig. 3 The comparison of averaged DSC curves for healthy control subjects (*continuous line*) and patients with early (*dash–dot*) and severe stage (*dotted line*) of COPD

with severe COPD is significantly shifted towards higher temperatures. Among parameters describing serum denaturation transition (shown in Table 1), only M_1 has been found statistically different for control and COPD groups ($p = 0.04$ in Kruskal–Wallis test). The highest value of $M_1 = 67.6 \pm 1.6$ has been obtained for patients with severe COPD. It is not possible to recognise in our study the effect of corticosteroids because all patients with severe COPD were treated with this medicine.

The results of our studies suggest age dependence of serum denaturation transition. The first moment M_1 of the thermal curve is significantly higher for patients above 55 years old (Table 2). However, four from five patients with severe stage of COPD have belonged to the age group above 55. Thus, the probable origin of ascertained differences may be disease development with age. On the other hand, Garbett et al. [8] found no remarkable differences for age in their DSC studies of plasma from patients with cervical cancer. In order to investigate the age dependence of serum DSC transition, the extended studies on healthy subjects should be done.

The alterations in thermal curves for individuals suffering from COPD are probably not only a consequence of changes in the concentrations of the major constituent serum proteins but can arise also from the interactions between these proteins as well as from changed fractions of ligated and unligated albumin. It was suggested earlier [7] that changes in DSC curve shapes and positions characteristic for disease state can be connected with the secretion into plasma of disease "biomarker" peptides that can bind to the most abundant plasma proteins, particularly albumin. These binding interactions can alter the thermal denaturation of serum proteins. The reported molecular size

Table 2 The comparison of thermodynamic parameters (mean ± standard deviation) of serum denaturation transition for two age groups (in parenthesis—the values found for averaged curves)

Serum of patients	T_m/°C	ΔH/J g^{-1}	HHW/°C	M_1/°C	C_p^{70}/C_p^{62}
Below 55	62.0 ± 0.2 (61.9)	21.9 ± 1.6 (22.0)	15.5 ± 1.3 (17.1)	**64.6 ± 0.4** (64.5)	0.63 ± 0.17 (0.63)
Above 55	66.4 ± 2.5 (62.0)	18.9 ± 0.7 (19.1)	15.1 ± 1.4 (18.3)	**67.7 ± 1.6*** (67.5)	1.19 ± 0.75 (0.97)

* Significant difference in comparison with control group ($p < 0.05$ in Mann–Whitney U test)

heterogeneity of some serum immunoglobulins in health and disease [9, 10] and the possibility of their migration between the monomeric and dimeric (or higher polymeric) state seems to be additional reason of differences between DSC profiles of serum from healthy and ill subjects.

Conclusions

The radically different serum DSC curves have been seen for patients with advanced stage of COPD in comparison with healthy controls from similar age group. Similarly, as for other diseases [6–8] a shift of the endothermic transition towards higher temperatures and a decrease of intensity at $\sim$62 °C with concomitant increase of intensity at $\sim$70 °C has been observed. Statistically significant increase of the first moment of the thermal curve with respect to the temperature axis has been found for severe state of COPD in comparison with normal serum. For early stage of disease similar direction of changes has been observed. However, further studies based on a more extensive set of COPD patients as well as healthy control are needed for establishing the possibility of COPD detection and monitoring by DSC.

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